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Experiments and Experiences in Biomanipulation Studies of Biological Ways to Reduce Algal Abundance and Eliminate Blue-Greens

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EXPERIMENTS AND EXPERIENCES IN BIOMANIPULATION - STUDIES OF BIOLOGICAL WAYS TO REDUCE ALGAL ABUNDANCE AND ELIMINATE BLUE-GREENS.

by

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R 803870
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16. ABSTRACT

Studies have been done to find alternatives to restoring or managing lakes by controlling external sources of nutrients. The guiding principle has been to understand and use biological interactions within lakes. This process is called biomanipulation and it is clear from the results that algal abundance and type can be varied substantially by one or more of the following procedures:

1. Elimination of benthivorous fish which recycle phosphorus from sediments.
2. Manipulations of algal populations by lowering pH, causing artificial circulation. and
3. Increasing abundance of larger herbivorous zooplankters by reducing predation on them, by eliminating planktivores entirely or, by providing refuges from planktivores.

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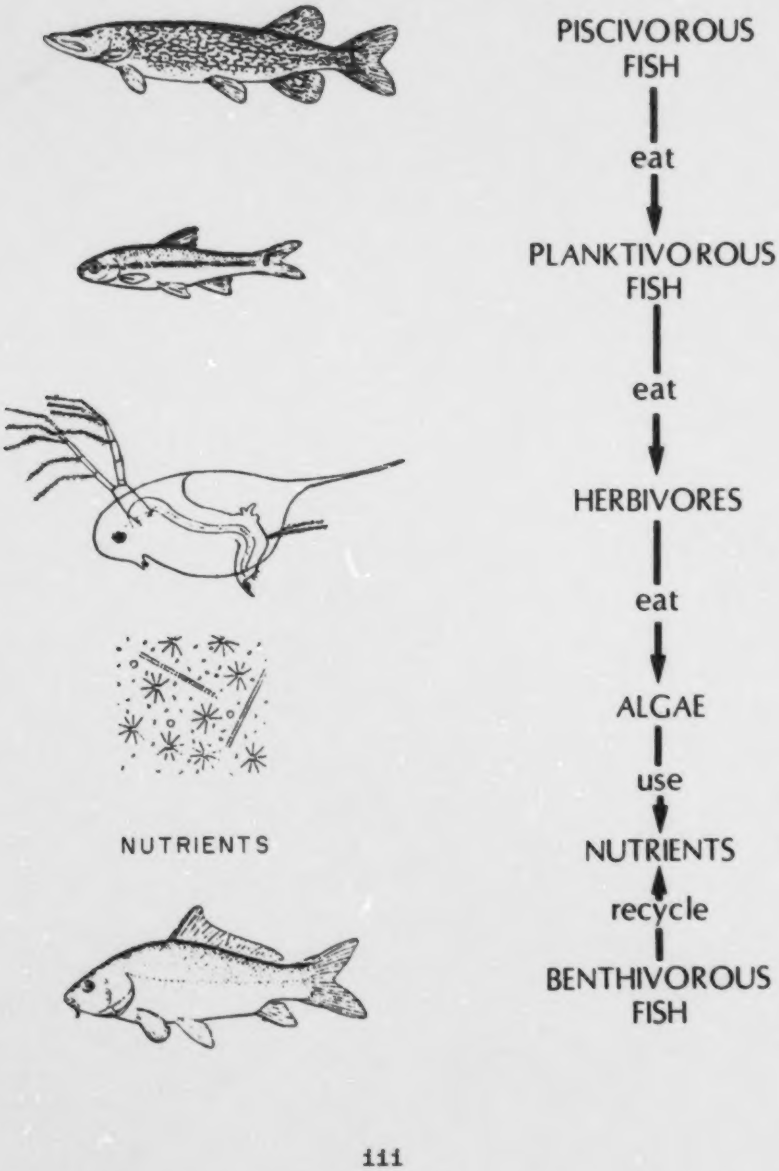
THE AQUATIC FOOD CHAIN

(Not to scale)


PISCIVOROUS FISH
eat
PLANKTIVOROUS FISH
eat
HERBIVORES
eat
ALGAE
use
NUTRIENTS
NUTRIENTS
recycle
BENTHIVOROUS FISH

ABSTRACT

Studies have been done to find alternatives to restoring or managing lakes by controlling external sources of nutrients. The guiding principle has been to understand and use biological interactions within lakes. This process is called biomanipulation and it is clear from the results that algal abundance and type can be varied substantially by one or more of the following procedures.

1) Elimination of benthivorous fish which recycle phosphorus from sediments.

2) Manipulations of algal populations by lowering pH, causing artificial circulation, or stimulating algal viruses.

3) Increasing abundance of larger herbivorous zooplankters by reducing predation on them, by eliminating planktivores entirely or, by providing refuges from planktivores.

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INTRODUCTION

For the last two decades or so limnologists appear to have been divided into two groups seemingly working in different directions -- the first to understand the basic physical, chemical, and biological processes operating in lakes, and the second to find ways of preventing, curing, or alleviating eutrophication of lakes. Those working in the first direction generally seem to look upon themselves as theoretical limnologists and by-and-large have left the "problems" of lakes to the second group. Those interested in eutrophication have for the most part taken a narrower view of lakes and have concentrated their efforts on identifying limiting or potentially limiting nutrients and their sources and on determining ways to reduce nutrient inputs or concentrations. This distinction has continued despite such early demonstrations as that by Hrbacek *et al.* (1961) that alterations in the fish populations could result in fewer algae; and that by Symons *et al.* (1969) that artificial circulation of stratified lakes could result in reduced abundance of blue-green algae. In other words it has long been known that key elements of eutrophic systems, indeed the very elements that are most undesirable -- abundant algae with their resulting decreased transparency, blue-green algae with their peculiarly undesirable characteristics -- are not strictly functions of nutrient inputs or concentrations. The possibility exists to alleviate the symptoms of eutrophication through avenues other than control of nutrient input from outside sources. The notable lack of exploration of such alternative courses probably resulted from at least three causes: the fact that Hrbacek *et al.* did not state their case forcefully; the fact that Symons *et al.*, and indeed many who used artificial circulation subsequently, were unable to explain their results; and the fact of the remarkable recovery of Lake Washington so energetically chronicled by Edmondson (1977). In effect nutrient manipulation seemed to work and the need for alternative measures to restore lakes may not have been recognized.

It was in this spirit that we approached our study of the Minneapolis Chain of Lakes (Shapiro and Pfannkuch 1973), a group of five lakes fed by urban storm drainage. To no one's surprise the eutrophic condition of these lakes was to a great extent a function of the large nutrient input. However, elimination of these inputs was and still is not possible for many reasons -- economic, energy requirements, etc. Also, various in-lake treatments such as nutrient precipitation were not likely to be of use because of the continuous nature of the nutrient inputs. Fortunately, at the same time that we were studying the lakes and beginning to appreciate these problems, we were doing other things. First, we were artificially circulating lakes, e.g. Lake Calhoun. From this we learned that circulation, although in this case it increased the abundance of blue-greens, can have biological effects of potential significance to lake restoration. Notably the circulation caused an increase in the number of large herbivorous zooplankters and this, as shown by Hrbacek *et al.* (1961), could result in fewer algae. Second, we were investigating the effects of increased carbon dioxide concentrations and of reduced pH on algal populations. From this we learned that either treatment leads to an increase in green algae at the expense of blue-greens, and we tentatively tied this to the sometimes success of artificial circulation. Third, we were studying the likelihood that fish removal by winter-kill or by fish toxicants could be beneficial because of removal of benthivorous fish, which we speculated might act as nutrient pumps (Lamarra 1975). Soon afterward we looked at the other possibility -- that fish removal might be beneficial also by allowing herbivorous zooplankters to increase in size and abundance. More recently in considering the mechanisms of circulation and the reasons for blue-green reduction we have studied the possible role of cyanophage.

Thus there developed the possibility that a complex of methods other than nutrient (N and/or P) manipulation per se could be used to help restore lakes. As the methods all involved manipulating the biota in various ways, the term "biomanipulation" was coined to describe the approach. Thus, biomanipulation is not a method -- it is an approach -- an attitude as it were. The main precept is that control of outside sources of nutrients is not the only way to restore lakes which are in a eutrophic condition, but that there exist within lakes biological phenomena that act to increase nutrient levels and to emphasize the effects of high nutrient levels and that if we understand the phenomena we can use them to our advantage.

This report is a compilation of studies we have made on these biological phenomena. They are, as with all ongoing research, in various stages of resolution. Some have been wholly funded by the U.S. Environmental Protection Agency; and some only partially, with the remainder coming from the National Science Foundation and the Department of the Interior. Our approach has been to use any and all methods to obtain information that could bear on the role of the biota in establishing conditions in lakes that would lead to methods for manipulating the biota in an appropriate fashion. Consequently, included in this report are:

- results of laboratory experiments,
- results of small-scale field experiments,
- results of large-scale field experiments,
- detailed observations on lakes unintentionally manipulated by winter-kill,
- detailed observations on lakes intentionally manipulated by fish toxicants,
- detailed observations on lakes not manipulated in any way,
- selected observations on lakes studied as part of a mass survey,
- selected observations on lakes from the files of the Minnesota Department of Natural Resources, the Minnesota Pollution Control Agency, and by others.

The manipulations to be described may be categorized as follows:

I. Those involving biological effects on the size of the nutrient pool.

II. Those involving algal abundance and type without regard to the nutrient pool. These include:

1. manipulations of the algae themselves
2. manipulations of zooplankters
3. manipulations of planktivorous fish
4. manipulations of piscivorous fish
5. manipulations of the physical environment.

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PROJECT SUMMARY

Introduction

Lake restoration ventures designed to reduce the abundance of undesirable algae almost always are based on the premise that reduction of nutrient input from external sources or from anoxic sediments is the key to success. Rarely have biological interactions within lakes been exploited deliberately to reduce or help in the reduction of such algal populations. However, consideration of such an approach, termed biomanipulation, as opposed to nutrient manipulation, indicates that it has great potential alone or in combination with nutrient manipulation.

Figure 1 shows some of the possibilities. Note that although the end goal is reduction of algal biomass, none of the possible manipulations involve nutrients directly. Most deal with changing the quantitative and qualitative relationships among the biota so that the desired end is achieved. It should be evident that while some of the possible manipulations are more likely to succeed than are others, most are likely to be more feasible and less expensive than direct reduction of the nutrients. What we do not know is the extent to which the manipulations can be successful, their duration of effectiveness, or their freedom from unexpected consequences.

This report is a summary of work done on biomanipulations at the Limnological Research Center up until 1980.

Among the possibilities for such manipulation that we have studied are:

I. Elimination of bottom-feeding fish which, through their feeding activities, increase the nutrient concentrations and thereby the abundance of algae in lakes in which they abound;

II. Manipulations of algal populations to change species composition and/or reduce abundance by (A) lowering pH, (B) causing artificial circulation, (C) stimulating activity of viruses that attack blue-green algae;

III. Direct manipulations of zooplankton populations to increase abundance of herbivorous species and therefore grazing on the algae;

IV. Indirect manipulations of zooplankton herbivores by manipulating their predators -- planktivorous fish -- by (A) experimental additions, (B) elimination of planktivores by rotenone treatment, and (C) elimination of planktivores by winter kill;

V. Modifications in oxygen concentrations that may lead to large changes in algal populations via their effects on refuges for zooplankters.

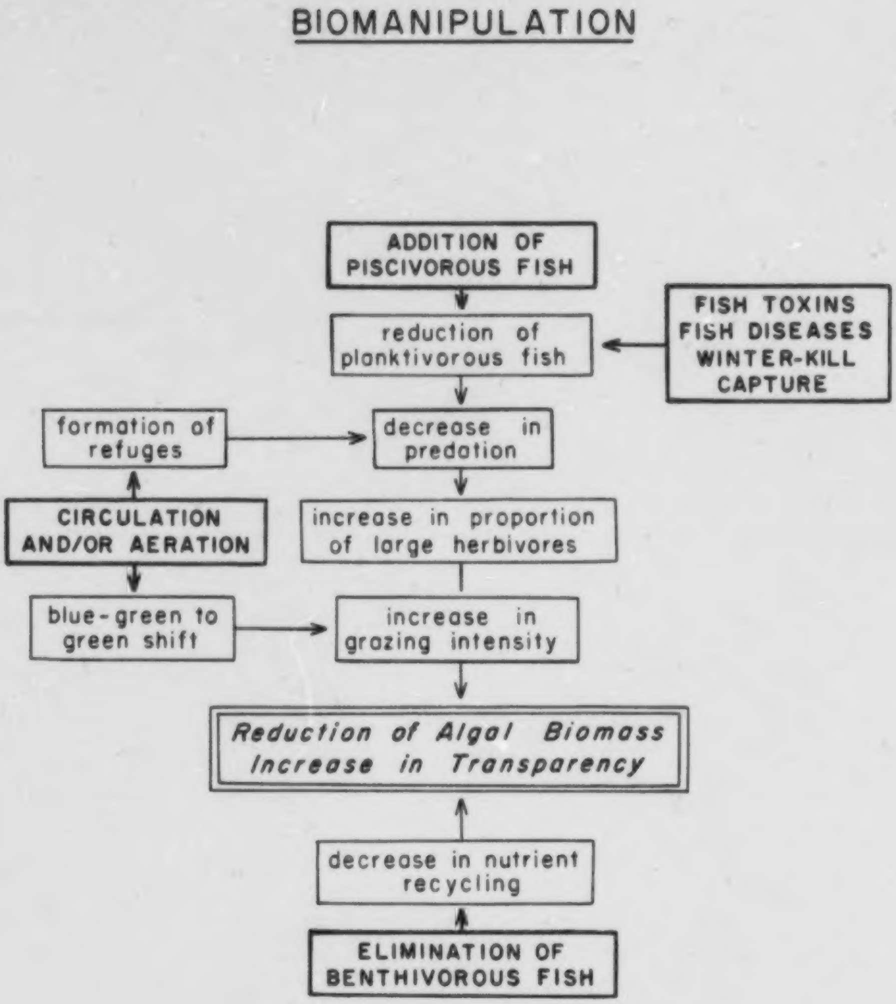
BIOMANIPULATION
ADDITION OF PISCIVOROUS FISH
reduction of planktivorous fish
FISH TOXINS
FISH DISEASES
WINTER-KILL
CAPTURE
formation of refuges
decrease in predation
CIRCULATION AND/OR AERATION
increase in proportion of large herbivores
blue-green to green shift
increase in grazing intensity
Reduction of Algal Biomass
Increase in Transparency
decrease in nutrient recycling
ELIMINATION OF BENTHIVOROUS FISH

Fig.1. Some aspects of biomanipulation.
The central goals of reduction of algal biomass, and increased transparency, are achieved through a variety of manipulations such as those shown in darker type. Mechanisms are indicated in lighter type.

I Elimination of bottom feeding fish

The work of Lamarra showed that bottom-feeding fish excrete phosphorus and nitrogen compounds and that the rate of excretion depends on the fish size, the temperature and the type of lake sediment. Lamarra hypothesized that such input could be a significant contribution to the total nutrient loading of lakes. An opportunity to test this idea arose when the Minnesota Department of Natural Resources decided to restructure the fish population in Lake Marion, a shallow (mean depth = 1.98 meters) large (172 ha) lake in south central Minnesota. Estimates of the fish population and its characteristics were made before the rotenone treatment using mark-recapture methods, and after the treatment using shore census of the dead fish. The latter method gave much higher values. Using these values, annual inputs for fish excretion were calculated at 88 mg/m2 per year for phosphorus and 270 mg/m2 per year for nitrogen. The Minnesota Pollution Control Agency had previously calculated the total phosphorus loading rate to the lake from its primarily agricultural watershed as 84 mg P/m2 per year. Therefore, the fish provide about half the phosphorus input to the lake. Based on two years' study of the lake before treatment, predictions have been made regarding reductions in algal biomass and productivity and increases in transparency expected from reduction in total phosphorus. Data to test these predictions have been collected and are in process of analysis.

II A Manipulation of algal populations by pH lowering

Preliminary studies had confirmed the hypothesis that green algae are favored over blue-greens at lower pH values. In these preliminary experiments, nutrients plus CO2, or nutrients plus acid, added to the waters of Lake Emily caused green algae to become dominant, while adding nutrients alone caused the blue-greens to increase. A total of 70 experiments have now been done in the field and in the laboratory and the conclusions are as follows:

a) The phenomenon is reproducible. In every case (19) in Lake Emily where CO2 was added with nitrogen and phosphorus, the blue-green to green shift occurred.

b) The phenomenon is not limited to Lake Emily. Ten other lake waters have been tested, and the shift took place in all of them.

c) Additions of HCl generally had the same effect as additions of CO2, but exceptions occurred.

d) The shift occurred whether field experiments were begun in June or late September.

e) Field experiments with pH controlled enclosures showed that the shift from blue-greens to greens occurred at pH values of 5.5 - 8.5, when CO2 was used, and at pH values of 5.5 - 7.5 when HCl was used.

f) In most of the experiments the green algae resulting from the shift were Scenedesmus and Chlorella, and in one experiment there were 22 species and subspecies of Scenedesmus.

g) The shift from blue-greens to greens seemed to be more rapid in spring and fall than in summer. This may be related to the size of the inoculum of greens as experiments with different sizes of inoculum showed the rate increased with a higher initial proportion of greens.

h) The shift often seemed to occur precipitously, and it involved almost all species of blue-greens in the lakes tested. However, some blue-greens remained, for if the pH was raised, they again began to regain dominance.

i) The reason for the shift is obscure. It may involve competition between the two types of algae, but the increase of the greens occurs after the decrease of the blue-greens. Thus the two phenomena are distinct. As the blue-greens disappear, phosphate and ammonia are found in the water, but disappear as the greens grow.

j) One possibility is that the blue-greens are affected by algal viruses at lowered pH. This is suggested by the manner in which the filaments break up. The role of nutrients appears to be important to the greens. If arsenate, which reduces phosphate uptake by the greens, is added the shift is delayed or prevented. Chlorine additions at high pH also cause the shift, presumably by a different mechanism.

II B Manipulation of algal populations by artificial circulation of lakes

Artificial circulation, frequently termed aeration, has been a lake restoration technique of limited value probably because of the lack of a proper theoretical framework for its use. To remedy this situation we constructed two such frameworks, one to explain the shift from blue-green algae to greens that is often observed, and one to explain the diminution in algal biomass that sometimes occurs. The hypotheses were tested in two lakes in a series of eight experiments utilizing a total of 76 enclosures that were 1 meter in diameter and extended from the surface through the thermocline to a depth of 8 meters. Some of the "bags" were open at the bottom while others were sealed and filled with surface water only, so that following temperature stratification in them by conduction the chemistry of their bottom waters could be adjusted. The enclosures were circulated by air to different depth and with different intensities and the results determined.

1. Species composition

Response of the phytoplankton at the species level appeared to depend primarily on changes in water chemistry in the euphotic zone during mixing. In the eutrophic lake with lower alkalinity, deep rapid mixing which increased nitrogen, phosphorus, and CO2 levels in the euphotic zone led to a shift from blue-green algae to greens and diatoms. Deep slow mixing, which also increased nitrogen, phosphorus, and CO2 levels in the euphotic zone resulted in increases in blue-greens. However, in the case of rapid mixing, carbon dioxide was introduced into the euphotic zone rapidly enough to lower pH values, while in the slow-mix enclosures pH remained high. This result is consistent with those of the previous sections.

Not only did the green algae benefit from rapid circulation, but diatoms also showed increases. As this occurred during shallow mixing as well, without the increase in nutrients and CO2, the mechanism must be different - possibly related to turbulence preventing the diatoms from sinking out of the euphotic zone.

Circulation in the higher alkalinity eutrophic lake was not as successful in shifting algal species composition. Not only was the water more buffered against pH change, but the concentration of CO2 in the hypolimnion was lower. Consequently, pH values did not decrease significantly during circulation. Furthermore the lake contained a metalimnetic population of Oscillatoria rubescens which generally increased in abundance in proportion to the total phosphorus increases in the euphotic zone resulting from mixing, and probably also as a result of increased light and temperature.

2. Community response

Data from the circulation experiments were also used to construct and test a mathematical model describing response of the total algal community. The most important variables in the model were found to be Zm, the mixed depth, and TP, total phosphorus, which have opposite effects on the maximum concentration of chlorophyll in the mixed layer during circulation. An increase in Zm causes a decrease in chlorophyll and an increase in TP causes an increase in chlorophyll. The relative magnitude of these changes therefore determines whether the chlorophyll concentration will increase or decrease. Furthermore the size of the chlorophyll change will be a function of such other factors as the N/P ratio, as at higher ratios the yield of chlorophyll/P is greater; the loss rate as a result of death, sinking, and grazing - factors also affected by circulation; and the extinction coefficient, Ew, of the water as it is determined by non-algal substances dissolved or suspended in it.

These results demonstrate why without an adequate theoretical framework it has been difficult to predict and/or understand the qualitative and quantitative changes that have occurred in lakes during circulation. The results obtained here will be useful in designing future attempts.

II c. Manipulation of algal populations through the use of specific viruses

Attempts have been made without success to control blue-green algae in lakes by utilizing the known capacity of several viruses to lyse them. As part of an investigation into the mechanism of the shift from blue-greens to greens at lowered pH, we studied, in the laboratory, the relationship between algal viruses and their hosts. We used the blue-green *Plectonema boryanum* and the Cyanophage LPP-1. Among the factors studied were: 1) the effect of pH alterations, 2) the effect of algal host age and density, 3) the effect of nutrient concentration, and 4) the effect of other algal species. The most relevant observation was that the alga thrives at both high (>10.5) and low (<7.5) pH values in the absence of the virus but it is lysed at the lower values in the presence of the virus. The implication is that lowering pH by artificial circulation of a lake may result in lysis of the blue-greens by viruses present in the system. However, more work needs to be done to determine whether this is what actually happens

III Direct manipulation of zooplankton populations

Decreases of algal abundance could result from increases in herbivore abundance. Therefore, experiments were conducted on the feasibility of using pantothenic acid, previously reported to be effective, to increase *Daphnia* abundance. Results were negative and it is concluded that such manipulations, including attempts to add herbivores directly to lakes, would be ineffectual. It is pointed out that certain pesticides may be exceedingly effective in eliminating *Daphnia*, however.

IV Indirect manipulation of zooplankton populations via planktivorous fish

A. Experimental

Experiments in which different densities of planktivorous fish were studied for their effects on zooplankton and algal populations were carried out in enclosures, divided ponds and whole ponds.

1. Enclosure experiments

In the enclosure experiments (1 meter diameter, 1 meter deep) additions of bluegill sunfish eliminated such herbivores as Daphnia pulex and Daphnia galeata while allowing the smaller species Daphnia ambigua and Daphnia parvula, to develop. Effects on the algae were dramatic with algal biomass in the enclosures with fish averaging, in one series, 16-fold that in the enclosures without fish. In some of the experiments, as fish predation intensity increased, filamentous blue-greens became relatively more abundant. In these experiments in the absence of fish predation the predominant algae were greens. The effects on algal biomass were the result of fish predation on the zooplankton, rather than fertilization of the enclosures by fish excreta. This was shown by experiments in which nutrients were added intentionally to the enclosures.

2. Divided pond experiments

These were done by dividing a small pond (0.5 ha) with polyethylene sheeting. One half contained numerous fathead minnows and the other half contained a few larger fish. As in the enclosures, Daphnia pulex was eliminated in the half containing the minnows and Daphnia ambigua and Daphnia parvula appeared. Consequently the algal biomass in this half of the pond averaged five times as high as that in the other half during July. This was not a result of greater phosphorus availability as the phytoplankton/P ratio was an average of 3.4 times as high in the "minnow" half.

3. Whole pond experiments

Two ponds side by side, which normally winter-killed, were used. One was stocked with mature perch and bluegill sunfish. One year later the ponds differed greatly in their zooplankton communities. In the stocked one, Daphnia pulex was absent, chlorophyll concentrations were high and transparency was low. The pond not stocked had large populations of Daphnia pulex, generally low chlorophyll concentrations and high levels of transparency.

During these investigations it was discovered that under certain circumstances the presence of Daphnia pulex appears to result in an abundance of Aphanizomenon flos-aquae in the form of large flakes not grazeable by the Daphnia. This has been noted in other studies as described later.

IV B. Manipulation of planktivore populations with fish toxins.

1. Previous experiences in Minnesota

Examination of the files of the Minnesota Department of Natural Resources revealed 13 lakes which had been treated in previous years with fish toxicants, and for which pre- and post-treatment transparency data were available. Of these, 7 had higher transparencies after treatment, 2 probably increased in transparency and 4 showed no change. In some of the lakes, the effect appeared to last for several years.

2. Effect of rotenone in Wirth Lake

This (16 ha, 4.3 m) lake is eutrophic from storm drainage input. Over a period of several years the lake received a variety of ameliorative techniques such as nutrient export, artificial circulation and piscivore stocking. However, beneficial effects were minimal (circulation actually increased nutrients and algal concentrations) until the lake was treated with rotenone in fall, 1977. In 1978, Daphnia pulex became abundant and, despite the circulation-caused high nutrient levels, it kept algal concentrations very low and transparency high until August. In August, Aphanizomenon flos-aquae became abundant in the flake form, disappearing only in September when Daphnia pulex were also absent. Evidence suggests that had the lake not been treated with rotenone, the piscivores would have controlled the planktivore populations and Daphnia pulex might have become abundant for this reason.

3. Effect of rotenone in Clear Lake

This small lake divided by a roadway was treated with rotenone after one year of study. The half which had not previously winter-killed was affected most by the rotenone treatment. Daphnia pulex became abundant and algal biomass declined sharply.

IV C. Manipulation of planktivores by winter-kill

1. Lakes affected in 1978-79.

Many Minnesota Lakes winter-killed in 1978-79. Nineteen lakes including non winter-kill controls were sampled four times during spring and summer of 1979 to determine the effects. Of eight lakes suspected of hard winter-kill, four had Daphnia pulex in them, and in three it was the dominant crustacean, averaging 19-33/l. Daphnia pulex also appeared in two lakes suspected of partial winter-kill and in one lake known to be low in panfish.

Chlorophyll/TP ratios in the four lakes with abundant D. pulex averaged less than .132 ± .046. Among the remaining fifteen lakes, chlorophyll/TP averaged .362 ± .136.

Transparencies of the four D. pulex lakes averaged greater than 2.07 ± .57 m and that of the remaining fifteen lakes 1.63 ± .68 m. For three control lakes, for which pre- and post-winter-kill transparency data were available, no transparency changes were noted following winter-kill, but for three partial winter-kill lakes, transparency doubled after the winter-kill.

With regard to the algal population, three of the four lakes in which D. pulex were abundant were characterized by an abundance of Aphanizomenon flos-aquae in its flake form.

2. Effect of winter-kill in Lake of the Isles

In 1976-77, Lake of the Isles in Minneapolis suffered a severe winter-kill. This storm drainage-fed eutrophic lake had perennially developed large crops of blue-green algae and low transparency during summer. In 1977, transparencies were so high that macrophyte problems prevailed requiring mechanical harvesting. The high transparencies were probably caused by grazing by Daphnia pulex which became abundant in the lake following the demise of the planktivores. At the same time as D. pulex appeared, D. magna was found in the lake, and D. galeata increased in size over previous years. Although some of the increase in transparency resulted from the decrease in chlorophyll, part of the increase rests on the fact that much of the remaining chlorophyll was present in Ceratium. Not only do these organisms not attenuate light effectively but they were most abundant at some distance below the lake surface.

V. Effects of physical-chemical conditions on algal populations

Lake Harriet (143 ha; 8.8 m mean depth) in Minneapolis perennially produces lower algal concentrations than expected from its nutrient concentrations. This discrepancy has been attributed to grazing by the abundant Daphnia galeata, and indeed low chlorophyll concentrations have been correlated with a high proportion of phaeophytin -- evidence of such grazing. In 1974, summer chlorophyll concentrations in the lake suddenly increased from their usual 5 ug/l to as high as 47 ug/l. Algal volumes increased in proportion and transparencies decreased. The situation ameliorated in 1975, and by 1976 was "normal". In recent years, the same phenomenon appears to be recurring.

The explanation for the high chlorophyll in 1974 appears to lie in the reduced numbers of Daphnia present that year. The decreased numbers of Daphnia may have resulted indirectly from the somewhat higher concentration of phosphorus in the lake in 1974. That is, we hypothesize that the increased phosphorus levels, too low to raise algal abundance by more than 20 or 30 percent, nonetheless allowed primary production in the euphotic zone (not measured) to increase to the extent that dissolved oxygen concentrations in the upper part of the hypolimnion (measured) became too low to allow the Daphnia to retain the zone as a refuge from fish predation. Consequently the Daphnia were forced to inhabit the waters above, where predation depleted their numbers and released the algal population from their herbivory. Hence the algal increase. If this hypothesis is correct it will represent the first true threshold effect of nutrients in stimulating algal biomass in a lake. It also opens the possibility that, if the upper portion of the hypolimnion of such a lake were to be oxygenated artificially, Daphnia could find a refuge from the fish, and remain abundant enough to limit the size of the algal population.

I. BIOLOGICAL EFFECTS ON THE SIZE OF THE NUTRIENT POOL. THE ROLE OF BENTHIVORES IN LAKE MARION, MINNESOTA.*

It has been observed that the removal of fish from a lake, either artificially with fish toxicants or naturally by winter-kill, often results in reduced phytoplankton abundance and increased transparency. This observation has been documented by Hrbacek et al. (1961), Schindler and Comita (1972), Stenson et al. (1976), and others. One possible explanation for this phenomenon follows from the work of Lamarra (1975a). Lamarra demonstrated that benthivorous fish regenerate considerable quantities of nutrients from the bottom sediments by their feeding and digestive activities. Removal of such fish could eliminate a source of internal nutrient loading to the lake and lead to lowered algal standing crops. Management of the fish community has been proposed as a technique to control algal abundance (Shapiro et al. 1975). Successful development of this technique will require detailed study of the mechanisms involved.

Andersson et al. (1978), like Lamarra, found higher levels of phosphorus and nitrogen in experimental enclosures containing benthivorous fish than were found in fish-free controls. However, the significance of benthivorous fish excretion in the nutrient budget of an entire lake has never been directly evaluated. In order to do this, an experiment was designed involving Lake Marion, in McLeod County, Minnesota. Lake Marion, in 1978, had a fish community dominated by benthivores such as black bullheads (*Ictalurus melas*) and carp (*Cyprinus carpio*). An opportunity to study the effects of removing these fish existed when the Minnesota Department of Natural Resources planned to treat the lake with the piscicide, rotenone, for recreational fisheries management purposes, in Fall 1978. However, unforeseen increases in the price of the rotenone caused a postponement of the treatment until Fall 1979. Thus, two seasons of field work (during 1978 and 1979) have been carried out on Lake Marion in order to establish baseline data and predictions against which the response of the lake, in 1980, to fish removal will be compared. Although completion of the experiment awaits the 1980 season, the results to date are presented below.

METHODS

Sampling Procedure. All water samples were taken during the mid-morning hours at a single station in Lake Marion located over the deepest area of the basin (Fig. 1).

Temperature and Dissolved Oxygen. Depth profiles of temperature and dissolved oxygen were obtained with a Yellow Springs Instrument Co. Model 54 ABP dissolved oxygen meter.

Transparency. Secchi disc measurements (SD) were taken using a 20 cm white disc.

Phosphorus. Total phosphorus (TP) in depth-integrated whole water samples was digested with potassium persulfate, and analyzed colorimetrically by the ascorbic acid method (APHA 1971). Dissolved phosphorus (DP) was measured on filtrates by the same method. Particulate phosphorus (PP) is obtained by difference. Orthophosphorus (PO4-P) was measured on undigested filtrates using the ascorbic acid method. All values reported represent the mean of at least three replicate analyses.

Nitrogen. Total nitrogen (TN) in depth-integrated whole water samples was analyzed by alkali-persulfate digestion, followed by nitrate reduction and colorimetric analysis as described by D'Elia et al. (1977). In order to avoid clogging of the cadmium columns, an ammonium chloride buffer solution was used during nitrate reduction in place of the boric acid buffer employed by D'Elia et al. This substitution was shown not to alter the results. Nitrate in filtered samples was analyzed colorimetrically by the cadmium reduction method (APHA, 1971). The values reported represent the sum of nitrate plus nitrite (NO3-N and NO2-N). Ammonia (NH3-N) in filtered samples was analyzed colorimetrically by the method of Chaney and Marbach (1962). All values reported represent the mean of at least three replicate analyses.

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Photosynthesis and Respiration. Rates of planktonic photosynthesis and respiration were measured in situ by the light and dark bottle oxygen method. Glass 300 ml B.O.D. bottles were filled with water from depths of 0,.5, 1.0,1.5,2.0, and 3.0 m and incubated at depth for the 6-hour period preceding solar noon. Oxygen changes in the light and dark bottles were analyzed by the azide modification of the Winkler method (APHA, 1971). Daily rates of photosynthesis and respiration were obtained by multiplying the 6-hour rates of gross photosynthetic oxygen production and respiratory consumption by factors of 2 and 4, respectively. Conversion of oxygen changes into units of carbon assimilation and loss was done by assuming a photosynthetic quotient of 1.2 moles of oxygen evolved per mole of carbon assimilated, and a respiratory quotient of 1.0 moles of carbon respired per mole of oxygen consumed (Strickland, 1960). Integral rates of photosynthesis (Aint) and respiration (Rint) (mgC m-2 day-1) were obtained by numerical integration of the photosynthesis-depth profiles. "Light-saturated" volumetric rates of photosynthesis (Aopt, mgC m-3 day-1) were obtained by using the maximum volumetric rate observed in the photosynthesis-depth profile.

Planktonic Biomass. Chlorophyll a concentrations (CHL) were determined with 90% acetone extraction of samples ground on glass fiber filters, followed by trichromatic spectrophotmetric analysis (Strickland and Parsons, 1968). No correction was made for the presence of phaeopigments. Particulate organic carbon (POC) was measured by the dichromate oxidation method (Strickland and Parsons, 1968). Because naturally-occurring particulate organic carbon is in a chemically more reduced state than the glucose used to calibrate the POC analysis, a conversion factor of .83 mg natural POC per mg POC as glucose was applied. This factor was obtained by assuming that the reduction state of naturally occurring POC reflected the photosynthetic quotient of 1.2. All CHL and POC values reported represent the mean of at least three replicate analyses.

Algae. Depth-integrated water samples were preserved in Lugol's solution for qualitative analysis.

Zooplankton. Depth-integrated zooplankton samples were obtained from single vertical tows with a Wisconsin plankton net (17 cm diameter opening, 64 mesh size). The animals were preserved in a sucrose-formalin mixture for microscopic identification and enumeration.

Fish Population Estimates. The abundance of bullheads and carp in Lake Marion was estimated by two methods.

1. A multiple-census mark-recapture estimate was made, using the results of nettings carried out on four dates in August and September, 1979. The fish were captured in trap nets set for 48 hours with their leads extending into shore. Captured fish were

marked by removal of the right pectoral fin, and were returned to the water at a location near the center of the lake. The population numbers were estimated for each species and for each size class within a species according to the method of Schumacher and Eschmeyer (1943).

$$N = \frac{\Sigma m^2 c}{\Sigma m r} \qquad (1)$$

N = the estimated population number
m = the cumulative number of previously marked fish
r = the number of marked fish recaptured on a given sample date
c = the total number of marked and unmarked fish captured on a given sample date
Σ indicates a summation over all sample dates

Separate estimates were made for two size classes of bullheads. It proved impossible to obtain a mark-recapture estimate of carp numbers because these fish rarely entered the trap nets.

2. The second method of estimation involved a count of dead fish washed ashore five days after the lake was treated with rotenone in October, 1979. Length intervals along the shore were marked off systematically around the entire lake. Complete counts were made of the fish within each interval, with the assistance of about 50 seventh-grade students and their teachers from the Hutchinson, Minnesota, Junior High School. By knowing the length of shoreline contained within these intervals (20% of the total shore length), the total number of fish on the shore could be estimated. This number is an underestimate of the true number of fish in the lake because an unknown fraction of the fish never came ashore. Separate counts were made for each species. Length-frequency distributions were also obtained on samples of dead fish in order to develop estimates of numbers in each size class. Using this information, along with length-weight relationships for the two species, it was possible to make separate biomass estimates for 17 weight classes of bullheads and 7 weight classes of carp.

RESULTS

Lake Marion is a large, shallow, eutrophic lake located within the watershed of the South Fork of the Crow River in McLeod County in south-central Minnesota (T. 115,116N,R. 29,30W). A map of the lake is given in Figure 1. Agriculture is the major land use in the Lake Marion watershed. The main (south) basin of the lake is 172 ha in surface area with a mean depth of 1.98 m, and is drained by a single outlet at the south end. Depth profiles of temperature and dissolved oxygen indicate that the lake does not stratify during summer. The small north basin (34 ha) is dominated by

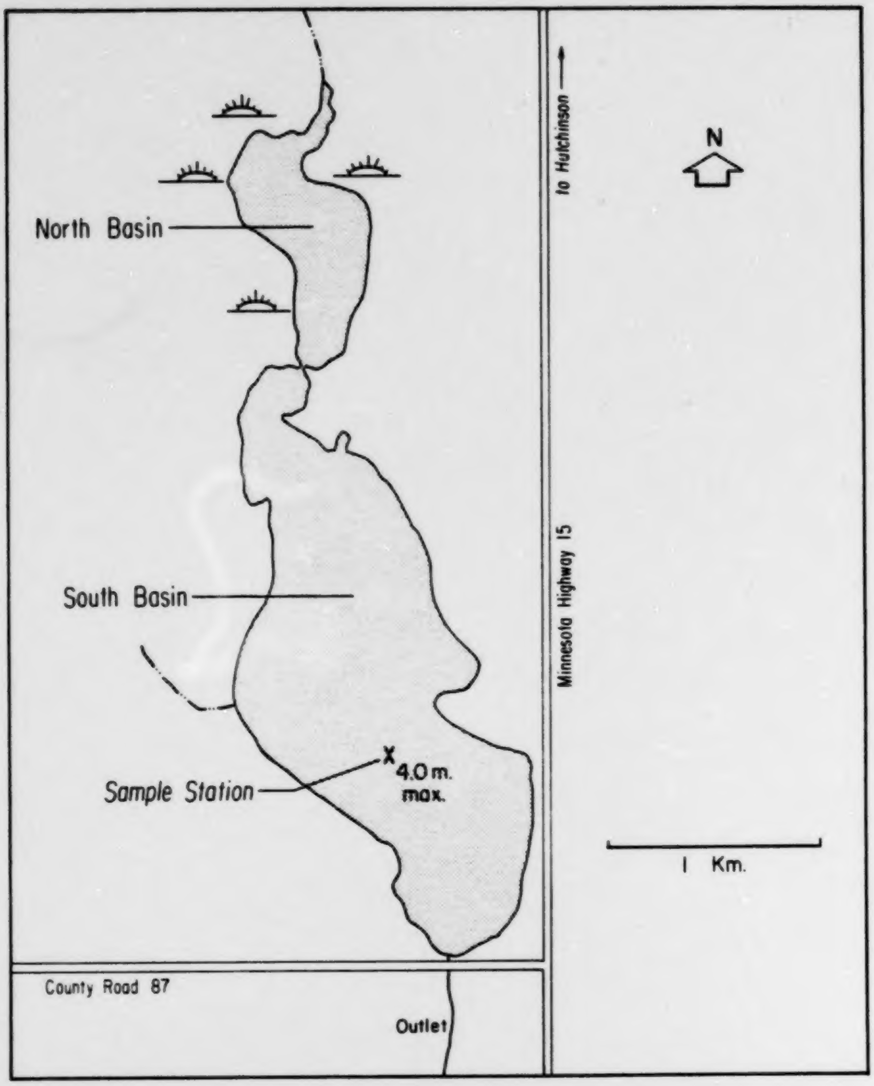
North Basin
South Basin
Sample Station
X 4.0 m. max.
to Hutchinson
N
Minnesota Highway 15
1 Km.
County Road 87
Outlet

Fig. 1 Map of Lake Marion, McLeod County, Minnesota.

emergent vegetation and drains into the main basin through a narrow culvert. The north basin was not sampled, and the results presented below apply to the main basin only.

Secchi disc transparency in Lake Marion was low, averaging .89 m in 1978 and .64 m in 1979 (June-September means). Algal biomass was moderately high during this period, with CHL averaging 36 mg m-3 in 1978 and 43 mg m-3 in 1979. Blue-green algae such as *Microcystis* sp. and *Oscillatoria* sp. were the dominant phytoplankton. Theoretical considerations (Brezonik, 1978; Megard *et al*., 1980) indicate that the reciprocal of the Secchi disc depth (SD-1) should be a linear function of the concentration of light-attenuating substances such as chlorophyll. This relationship holds true for Lake Marion in 1978 and 1979 as can be seen in Figure 2. The intercept of the regression of SD-1 on CHL can be interpreted as the average amount of "background" attenuation by materials not associated with the algae. When this intercept, with a value of .41 m-1 is compared with the average value for SD-1 of 1.40m-1, it can be seen that most (71%) of the light attenuation was caused by materials directly associated with the algae.

The suggestion that increased transparency and reduced algal abundance following fish kill are a result of reduced nutrient loading by benthivorous fish excretion carries with it the assumption that algal biomass and productivity are regulated primarily by nutrient concentrations. Plots of CHL and Aopt vs. TP and TN for Lake Marion (Figs. 3 and 4) give no apparent relationships, within seasons, between the nutrients and algal biomass or productivity. However, factors such as sampling error, temperature variations, and time lags in the response of the algal community to changes in nutrient levels could obscure such relationships. Better results have been obtained when season means of CHL and Aopt, for a large number of lakes, are plotted against TN and TP (Sakamoto, 1966; Dillon and Rigler, 1974; Jones and Bachman, 1976; Smith, 1979). The implication of these relationships is that nutrients such as nitrogen and phosphorus exert primary controlling influence on algal biomass and productivity. Figures 5 and 6 show that season mean values from Lake Marion fit well when compared with these established relationships. It is therefore reasonable to predict that a reduction in nutrient loading to Lake Marion, and a corresponding drop in nitrogen and phosphorus concentrations, should lead to reduced average summer algal biomass and productivity.

One way of determining the effect of benthivorous fish on levels of nutrients is to correlate past changes in fish abundance with changes in nutrient concentrations. Table 1 summarizes all the data that is available for Lake Marion. The period of 1975-1979 was a time of low winter dissolved oxygen concentration, chronic winter-kill, and dominance of the fish community by black bullheads. The sharp decline in bullhead abundance between 1977 and 1979 was probably a consequence of winter-kill conditions. The bullhead decline was accompanied by a pronounced drop in TP and a small drop in TN. This trend, though somewhat circumstantial, provides evidence that nutrient levels in Lake Marion may depend on benthivorous fish abundance.

A second way of determining the significance of benthivorous fish in the nutrient budget of Lake Marion is to compute the quantities of phosphorus and nitrogen excreted by these fish. To do this, the following information was assembled.

1. The specific excretion rates of nitrogen and phosphorus (ug/g wet wt/hr) by carp and black bullheads were obtained from the data of Lamarra (1975b). By measuring specific excretion rates of fish taken from Union

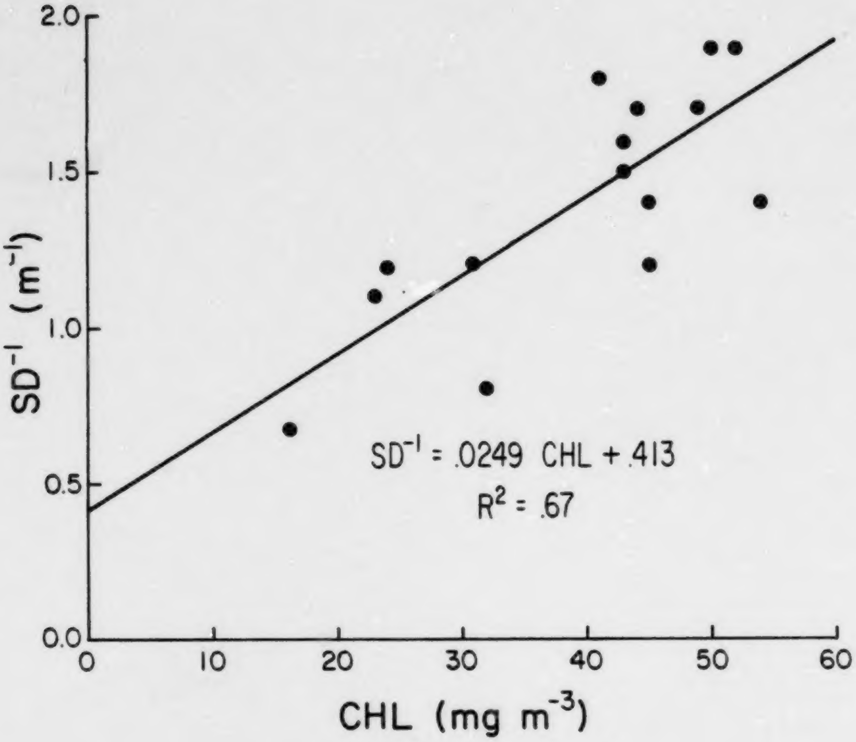

Fig. 2 The relationship between SD^{-1} and CHL in Lake Marion, 1978 and 1979.

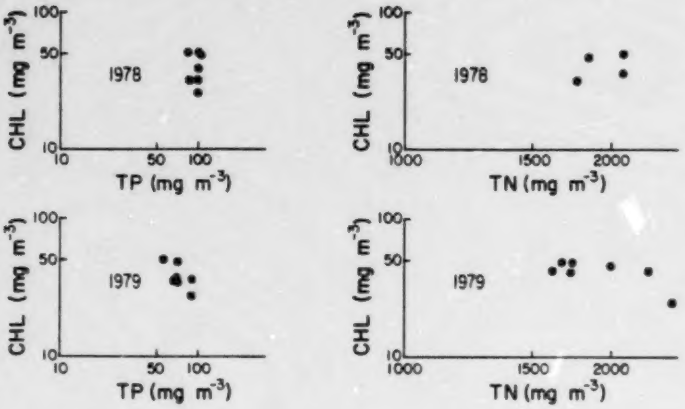

Fig. 3 The relationships between CHL, TP and TN in Lake Marion within the 1978 and 1979 seasons.

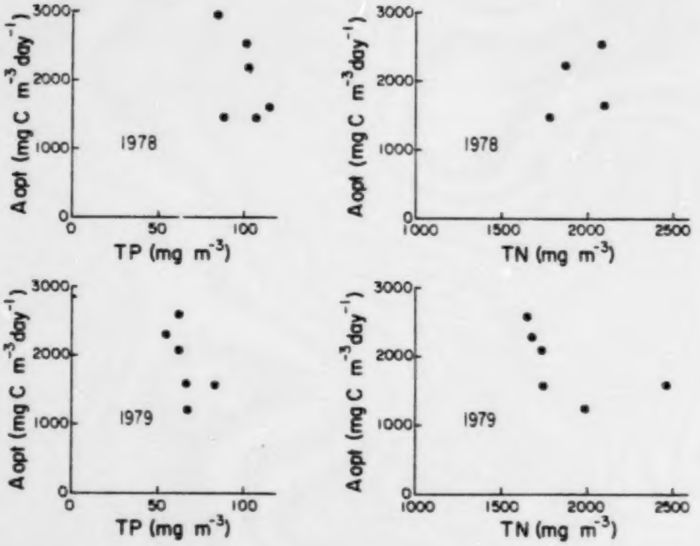

Fig. 4 The relationships between Aopt, TP and TN in Lake Marion within the 1978 and 1979 seasons.

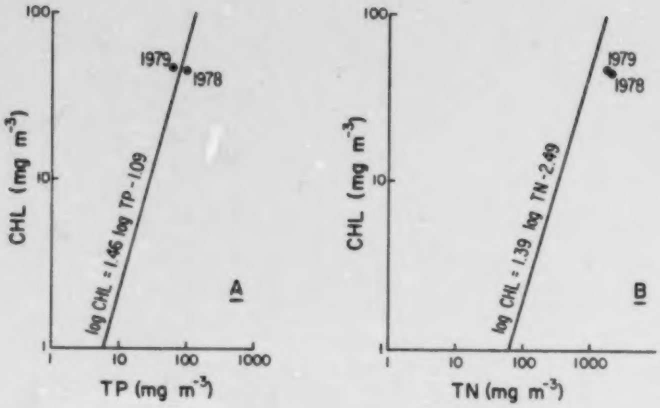

Fig. 5 July-August means of CHL, TP and TN in Lake Marion, 1978 and 1979, compared with the empirically established relationships of A. Jones and Bachman (1976), and B. Sakamoto (1966). The regression equation for CHL vs. TN was obtained from Sakamoto's Fig. 4.

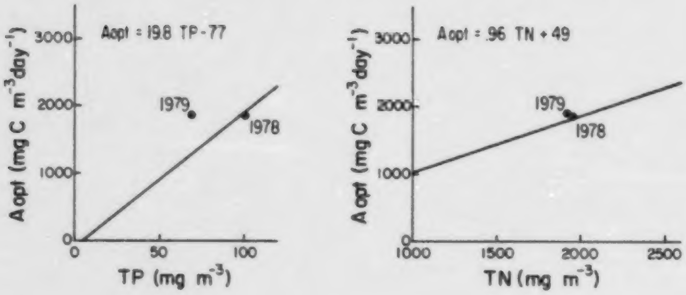

Fig. 6 June-Sept. means of Aopt, TP and TN in Lake Marion, 1978 and 1979, compared with the empirically established relationships of Smith (1979).

Table 1. Correlation of past changes in relative bullhead abundance in Lake Marion with changes in nutrient concentrations (mg m^{-3}). Bullhead values represent the mean number of bullheads captured per trap net per day of set.

Year	Relative Bullhead Abundance	TP	TN	Source
1975	---	93	1920	Minnesota Pollution Control Agency. Single sample date Sept. 1975.
1977	152	--	--	Minnesota Dept. of Natural Resources. Mean of 5 trap nets on a single August sample date.
1978	---	107	1960	Present study. July - Sept. means.
1979	60	63	1760	Present study. Nutrient values are July - Sept. means. Bullhead number is the mean for 22 nets distributed over 4 Aug. - Sept. sample dates.

Lake, Minnesota, and placed in small plastic enclosures for one-hour time intervals, Lamarra developed the following regression equations for carp.

$$\log_{10} E(DP) = -.49 \log_{10} W + .027 T + .77 \qquad (2)$$

$$\log_{10} E(NH3) = -.38 \log_{10} W + .04 T + 1.03 \qquad (3)$$

$E(DP)$ = specific excretion rate of dissolved phosphorus ($\mu g\ g^{-1}\ hr^{-1}$)
$E(NH3)$ = excretion rate of ammonia
W = wet weight of fish (g)
T = temperature (^{o}C)

Lamarra's specific excretion rate experiments on black bullheads were not extensive enough to permit the inclusion of temperature as a second variable in the regressions. However, by assuming the same temperature coefficient for black bullheads as for carp, the following equations for black bullheads can be obtained from Lamarra's data.

$$\log_{10} E(DP) = -.347 \log_{10} W + .027 T + .344 \qquad (4)$$

$$\log_{10} E(NH3) = -.701 \log_{10} W + .04 T + 1.08 \qquad (5)$$

It will be assumed that other variables affecting the specific excretion rates, such as sediment phosphorus content, were similar in Lake Marion to levels found in Lamarra's Union Lake.

2. In order to simplify calculations of loading rates, the year was divided into two 6-month seasons, summer and winter, during which it was assumed that representative water temperatures were 20 and 4 degrees C, respectively.
3. The biomass of benthivorous fish in Lake Marion in 1979 was estimated by the mark-recapture and the shore census methods. The results are shown in Table 2. The sharp discrepancy between the bullhead estimates by the two methods is disturbing. There are some possible reasons for the systematic error in the mark-recapture process which may have led to the underestimate, but they will not be discussed here. The results of the shore census will be used for the purpose of calculating nutrient loading to Lake Marion by benthivorous fish, although as noted before, this is an underestimate of the true biomass of fish in the lake.

Annual rates of nutrient loading by benthivorous fish excretion were then estimated as follows. Specific excretion rates for each weight class of the two species were calculated using Lamarra's equations, the mean weight of individuals in each weight class, and the temperature during each season. Loading rates for each weight class at each temperature were calculated as the product of the specific excretion rate and the total biomass of the weight class in the lake. These loading rates were summed over all weight classes and temperature periods to obtain the total annual nutrient loading rates by benthivorous fish of each species. The results are given in Table 3.

DISCUSSION

Bottom-feeding fish such as carp and bullheads are commonly found in lakes with obvious manifestations of eutrophication. Moyle (1968) noted a correlation between the presence of "rough fish", algal blooms, and low transparency in Minnesota lakes. The cause and effect relationships are not obvious however. One interpretation is that bottom-feeders promote water fertility by "stirring up" nutrients from the sediments. Lamarra (1975a) disagreed with this interpretation and demonstrated that stirring of the sediments did not produce increases in total phosphorus and chlorophyll the way fish activity did. A second mechanism linking benthivorous fish to turbidity might be the resuspension of light-scattering particles from the bottom by fish movements. In Lake Marion, the strong dependence of light attenuation in the water column on chlorophyll concentration (Fig. 2) is evidence that factors other than algae are of minor importance in causing low transparency. If benthivorous fish are, in fact, partly responsible for the eutrophic condition of Lake Marion, then the causal mechanism is probably nutrient regeneration by excretion, as suggested by Lamarra, rather than the stirring up of sediments.

In order to judge the significance of benthivorous fish in the overall nutrient budget of Lake Marion, the calculated nutrient loading rates from fish excretion must be compared with other sources of loading including external loading from the watershed, precipitation, and direct release from the sediments. Benthivorous fish excretion supplied phosphorus to Lake Marion, in 1979, at a rate of approximately 88 mg m-2 yr-1 (from Table 3). An estimate of phosphorus loading from external sources can be obtained from the Minnesota Pollution Control Agency (MPCA, 1975, unpublished report). The MPCA estimated phosphorus loading rates to Lake Marion from external sources, including drainage, direct precipitation, and septic tank seepage. The necessary values for nutrient inputs from the various sources and for the hydrologic factors were obtained indirectly from regional averages. The MPCA estimated that the external phosphorus loading rate to Lake Marion was about 84 mg m-2 yr-1. Apparently, fish excretion was as important as external sources in supplying phosphorus to the lake.

To determine whether fish excretion represents a significant fraction of the total loading, it is necessary to evaluate the rate of direct sediment phosphorus release. Values reported in the literature for phosphorus release rates from aerobic lake muds under laboratory conditions vary over a range of -2.0 - 9.4 mg m-2 day-1 (Kamp-Nielsen, 1974; Banoub, 1975; Ryding and Forsberg, 1977). This range is too broad to develop a reliable approximation for phosphorus release rates from Lake Marion sediments. For example, use of these literature values suggests that direct sediment release could be a considerable proportion of the total loading, even under aerobic conditions. Thus, Ryding and Forsberg (1977) quote a range of 0.3 - 2.0 mg m-2 day-1 for sediments derived from three shallow, highly eutrophic Swedish lakes. When expressed on an annual basis, this range of 110 -730 mg m-2 yr-1 can be compared with the loading rates to Lake Marion from benthivorous fish (88 mg m-2 yr-1) and from external sources (84 mg m-2 yr-1). In this manner one can speculate that direct sediment release of phosphorus was of such overwhelming importance that removal of benthivorous fish might have no significant impact on phosphorus levels in the lake.

On the other hand, Carlson (1975) measured phosphorus release rates from

Table 2. Estimates of Benthivorous Fish Biomass in Lake Marion, 1979.

Method	Species	Total Number	Total Wet Weight (kg)	Areal Density (kg ha^{-1})
Mark - Recapture	Bullheads	25,050 (23,090 - 27,400)*	1,860	11
Mark - Recapture	Carp	?	?	?
Shore Census	Bullheads	145,800	10,100	59
Shore Census	Carp	18,700	7,300	43

* Precision limits given for the mark - recapture bullhead estimate are the 95% confidence intervals, calculated according to Ricker (1975).

Table 3. Estimates of nutrient loading rates to Lake Marion during summer 1979 by benthivorous fish excretion.

	LOADING RATES			
	Phosphorus		Nitrogen	
Species	kg yr^{-1}	mg m^{-2} yr^{-1}	kg yr^{-1}	mg m^{-2} yr^{-1}
Bullheads	103	61	202	117
Carp	46	27	263	153
TOTAL:	149	88	465	270

aerobic sediment obtained from eutrophic Cottonwood Lake, Minnesota, a lake similar in morphometric and biological characteristics to Lake Marion. Carlson found no net phosphorus release from aerated muds. It is conceivable, therefore, that benthivorous fish excretion may indeed have supplied as much as half the total loading of phosphorus to Lake Marion. It may be of significance that the laboratory phosphorus release rates reported by Ryding and Forsberg (1977) greatly underestimated the net flux of phosphorus from their sediments _in situ_, as calculated from the nutrient budgets of their lakes. This discrepancy suggests that biological processes such as benthivorous fish excretion, that are not duplicated under laboratory conditions, may be of considerable importance in the internal phosphorus loading of their lakes as well.

The effect of removing the portion of the phosphorus loading derived from fish excretion can be analyzed by modifying Vollenweider's (1969) phosphorus balance model to account explicitly for internal loading.

$$dTP/dt = \frac{L(E) + L(F) + L(S)}{\bar{z}} - \sigma TP - \rho TP \qquad (6)$$

where dTP/dt = the change in total phosphorus concentration per unit of time (mg m-3 yr-1).
$L(E)$ = the areal rate of phosphorus loading from external sources (mg m-2 yr-1).
$L(F), L(S)$ = areal rates of internal phosphorus loading from fish excretion and from direct sediment release, respectively (mg m-2 yr-1).
TP = concentration of total phosphorus (mg m-3).
$\bar{z}$ = mean depth of the lake (m).
σ = specific phosphorus sedimentation rate (yr-1).
ρ = hydraulic flushing rate (yr-1).

Under steady-state conditions ($dTP/dt = 0$), equation 6 can be solved for the equilibrium phosphorus concentration (TP^*) as follows.

$$TP^* = \frac{L(E) + L(F) + L(S)}{\bar{z}\,(\sigma + \rho)} \qquad (7)$$

Of the terms in equations 6 and 7, L(S) and have yet to be measured in Lake Marion. Field studies of sediment release and phosphorus sedimentation rates are planned for Lake Marion in 1980 in order to evaluate one or both of these unknowns. When this is accomplished, it will be possible to use an approach similar to that of Dillon and Rigler (1975), with equation 7, to predict the decline in TP levels to be expected after the elimination of benthivorous fish excretion. Such a prediction can be verified by observing the lake's response in 1980 to fish removal.

Reductions in algal biomass and productivity can be expected if declines in TP levels do, in fact, occur. This prediction depends on the assumption that phosphorus, rather than nitrogen, is the variable controlling Chl and Aopt. This assumption is justified, given the observed mean TN/TP ratios of 18.4 and 28.4 in 1978 and 1979. These ratios indicate phosphorus limitation, according to Sakamoto (1966) and Smith (1979). The input ratio of nitrogen (NH_3)/phosphorus (DP) by benthivorous fish excretion is 3.1 (see Table 3). Therefore, elimination of the fish should produce an even greater degree of phosphorus limitation in Lake Marion.

There is one factor that could cause a delay in the expected nutrient decline in the lake following benthivorous fish removal. This is the potential for fish carcasses to release nutrients upon decomposition. Bull and Mackay (1976) indicated that carp contain about 0.5% phosphorus and 2.6% nitrogen, on a wet weight basis. By assuming that these values are approximately true for both carp and bullheads, and by using data given in Table 2, it can be calculated that decomposition of the carp and bullheads would release 52 kg of phosphorus and 435 kg of nitrogen. These quantities are significant when compared with the amounts of the nutrients excreted by the fish when they were alive (see Table 3). As a consequence, nutrient levels may not decline as rapidly as expected. However, inputs of nutrients from fish decomposition constitute a single pulse of loading, in contrast to excretion, which was a chronic source. Rapid sedimentation in the shallow Lake Marion basin should insure that the effects of this pulse are short-lived.

In addition to the elimination of benthivorous fish excretion, there is a second mechanism that could explain reduced algal abundance following fish kill. Planktivorous fish exert size-selective predation pressure on the zooplankton community (Brooks and Dodson, 1965), and may prevent the dominance of large-bodied species. Many investigators, including Hrbacek and Novatna-Dvorakova (1965), Galbraith (1967), and Stenson (1972, 1976) have documented the occurrence of numerous, large, herbivorous zooplankton such as Daphnia sp. following fish kill, and some have linked reduced algal densities in these situations to increased grazing losses. Addition of planktivorous fish such as the fathead minnow (Pimephales promelas) can reverse the effect, causing increased algal abundance, as shown by McNabb (1976), Helfrich (1976), and Lynch and Shapiro (1980). Fathead minnows were the dominant planktivore in Lake Marion in 1978 and 1979, and their removal by the rotenone treatment should result in greater zooplankton abundance and increased average body size of the zooplankters. Once the response of Lake Marion in 1980 to fish kill is known, it will be possible to study the significance of the grazing mechanism by observing changes in the zooplankton community and by evaluating the role of grazing losses in regulating algal biomass and growth rates. It will also, of course, be possible to verify the expectations about the significance of benthivorous fish excretion in the nutrient budget of Lake Marion.

All data collected on Lake Marion during the course of this project in 1978 and 1979 are tabulated in the Appendix (Table 3a).

Table 3a (Appendix) Measurements on Lake Marion in 1978 and 1979.

Date	Temp* (°C)	SD (m)	Chl (mg m-3)	POC (mg m-3)	Aopt (mgC m-3 day-1)	Aint (mgC m-2 day-1)
6/10/78	20.8	.9	23	5900	--	--
6/17/78	20.8	1.5	16	3400	719	1360
6/27/78	22.6	1.2	32	3140	1440	2930
7/8/78	22.4	.7	54	--	2980	2550
7/14/78	23.2	.6	49	5023	2544	2600
7/23/78	22.4	.8	45	--	2220	2410
8/4/78	21.6	.8	31	4840	1480	2190
9/7/78	24.1	.6	41	7860	1640	1810
5/1/79	9.9	.8	57	--	--	--
6/11/79	19.2	.65	43	--	--	--
6/29/79	20.9	.84	24	378	1560	2220
7/16/79	24.6	.64	43	4690	2590	2960
8/1/79	23.7	.53	52	4880	2290	2130
8/15/79	19.9	.70	45	--	1220	1480
9/3/79	22.4	.62	44	4370	2080	2020
9/14/79	16.8	.53	50	4620	1560	1640

* Average temperature in water column.

Table 3a (Appendix) continued

Rint (mgC m-2 day-1)	TP (mg m-3)	DP (mg m-3)	PO4-P (mg m-3)	TN (mg m-3)	(NO3+NO2)-N (mg m-3)	NH3-N (mg m-3)
--	96	40	-	--	--	--
1560	--	--	-	--	--	--
--	89	30	7	--	--	--
2490	85	28	9	--	125	--
1920	101	25	5	2080	13	19
1245	103	34	-	1870	8	0
1350	107	78	3	1790	2	0
1230	116	32	1	2100	2	5
--	83	--	-	2580	--	--
--	78	--	-	2310	--	--
1740	82	19	-	2460	--	--
2820	64	22	-	1650	--	--
2490	55	12	-	1690	--	--
1860	68	19	-	1990	--	--
2240	64	16	-	1740	--	--
1910	66	16	-	1740	--	--

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II. ALGAL MANIPULATIONS

IIA. THE EFFECTS OF PH AND CARBON DIOXIDE ON ALGAL COMMUNITIES*

In 1973 Shapiro described experiments, done in 1971 and 1972, in which he had caused shifts in algal populations from predominantly blue-greens to greens simply by adding nutrients and carbon dioxide to the systems. Because of the potential to use such shifts as a lake restoration technique, as well as because of the theoretical implications, it was desirable to learn more about the phenomenon. For example, does it occur only in Lake Emily, the original test site? How reproducible is it? Is it reversible? At what pH values does it occur? Are there resistant species? Above all it was desirable to learn the mechanism of the shift -- what causes it? It was to answer these questions that further experiments were done between 1972 and 1976.

METHODS

The early experiments had been done in the field in plastic "bag" enclosures. Because of their success, and our desire to maintain nearly natural conditions, most of the work to be described was done similarly. The physical setup was that shown in Figure 7. The "bags", of six mil polyethylene, were made by cutting appropriate lengths of extruded tubing, approximately 1 meter in diameter, and sealing the bottom ends. Heat sealing was unsatisfactory so the bottoms were sealed by folding over five centimeters, taping with waterproof "Griffolyn" tape, and folding and taping twice again. The tops of the bags were held open by folding the plastic outward over a 1-meter diameter hoop of 1-inch polyethylene tubing. Generally, to fill the bags they were taken about 20-30 feet from the raft and towed, by means of a three-point harness on the top hoop, toward the raft. In this way they filled with water from the first meter. They were then pushed under the outer rim of the outrigger, raised up, and the hoops fixed by nails to wooden blocks about 4-5 inches above the water surface. No metal was allowed to remain in contact with the water in the bags. As the rafts were east-west oriented and the bags were on the south side, no shading occurred. Generally the bags were about 1.5 meter deep and thus contained about 1 cubic meter of water. On occasion certain bags were filled by pumping water through a 2-inch diameter gasoline-operated pump that was first flushed. Usually the bags were topped off using the pump or a plastic bucket, and on occasion bricks wrapped in polyethylene were put in the bags to keep the bottoms down. Sampling was done by dipping from the surface after the first meter or so was well mixed with a wooden canoe paddle. Care was taken to not disturb the sedimented material on the bottoms of the bags. Nutrients were added in solution. Phosphorus was added as $KH2PO4$, generally over the first five days to a final concentration of 100 micrograms P/liter, and nitrogen was added similarly as $NH4NO3$ to a final concentration of either 700 or 1000 micrograms N/liter. pH was adjusted either by adding dilute HCl, dilute NaOH, or by bubbling in 100% carbon dioxide gas. Other additions will be described later. pH measurements were made with a variety of field instruments.

Most of the work was done on Lake Emily, which is, as noted in the earlier paper (Shapiro, 1973), a small lake just north of St. Paul, Minnesota. The lake is shallow for most of its expanse and many of the experiments were performed near the shallow southern end. However, the experiments at different pH values were done at the northern end in about 4-5

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*by Joseph Shapiro, Vincent Lamarra and George Zoto

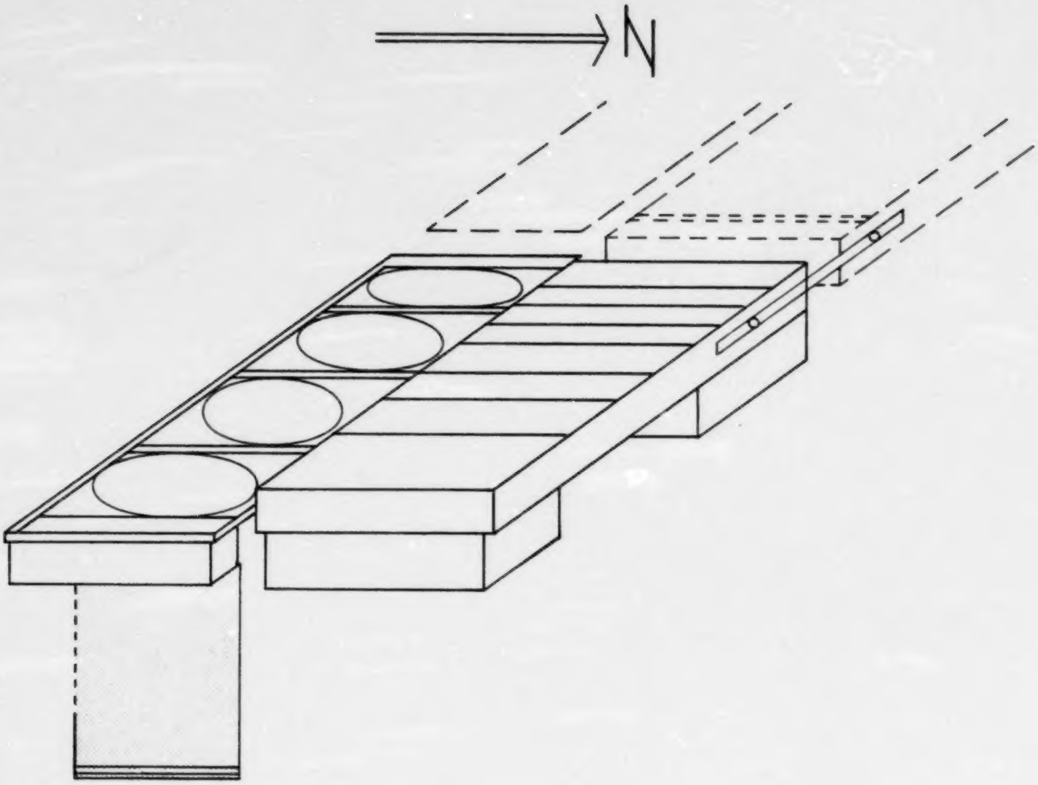


Fig. 7 Physical arrangement used in the enclosure studies.

meters of water. The lake is exceedingly eutrophic, being fed by storm drainage from the surrounding suburban areas.

RESULTS

Reproducibility

Although the carbon dioxide-induced shift had been demonstrated first in 1971 and confirmed in 1972, every time a series of experiments was done at least one pH 5.5 bag, continuously bubbled with CO2 and with nutrients added, was used along with an appropriate control with nutrients only to confirm the continued capacity of the population to shift. Such experiments are noted by asterisks in Table 4.

Of these 20 comparable lake experiments using additions of CO2 to pH 5.5, and additions of N and P, 19 showed the shift from blue-greens to greens. The sole exception was experiment F19 in which N and P were added six days after the CO2 treatment was begun. In some of the experiments stirring with air was continuous while in others stirring occurred only at sampling times. No difference in result was noted. It appears therefore that, as the controls never went over to greens and the CO2/nutrient treatments virtually always did, the phenomenon is highly reproducible.

On one occasion (Table 4, J(1)6) CO2 treatment was continued only long enough (about 30 minutes) to lower the pH to 5.5, whereupon the CO2 was turned off. In this case only a partial shift occurred.

Tests in Other Lakes

The phenomenon appears not to be limited to Lake Emily. On several occasions (H, I, J2) various other lake waters were tested by incubating them in the laboratory (300 foot candles continuous light, 22 degrees C) with N and P and CO2. Lake Emily water was treated similarly for comparison. Not only did the shift occur in Lake Emily under the laboratory conditions, but it took place in all 10 other lake waters tested (Table 4). In 3 of these tests the controls, containing N and P only, also changed. This may have been caused by CO2 leaking into the incubator from those systems being bubbled with it. They represent the only controls that shifted during all of the studies.

Time of Year

In addition to the phenomenon being reproducible, and applying to other lake waters and their algae, it appears to extend over most of the growing season for the algae. Experiments in Lake Emily have been begun as early as June 24 and as late as September 24, and have gone on as late as October 25, all successfully.

Effect of HCl versus CO2

Because one effect of the CO2 addition is to lower the pH to about 5.5, and because an understanding of the mechanism of the shift necessitates knowing whether lowering the pH artificially without CO2 addition is effective, three field experiments (Table 4, C(1) 5, 6; and D11) were done in which N and P were added and the pH was lowered to 5.5 with HCl. The shift took place in all three. However, in two laboratory tests with water from Quarry Lake the shift did not occur (Table 4, I).

Experiments at Different pH Values

The original intent of the work had been to test a hypothesis based on a publication of King (1970) on the relative ability of green and blue-green

TABLE 4

Experiments in which either CO_2 or HCL was used to lower the pH to 5.5 - 6.0. Experiments marked * are those where CO_2 and N & P were added to enclosures in the field.

SERIES & EXPERIMENT	DATE	LAKE	AGENT	NUTRIENTS	SHIFT	COMMENTS
* B 2	7/29/71	Emily	CO_2	+	Yes	
B 3	7/29/71	Emily	CO_2	-	Partial	
$C_{(1)}2$	7/23/72	Emily	HCl	-	No	
$C_{(1)}5$	7/23/72	Emily	HCl	+	Yes	
$C_{(1)}6$	7/23/72	Emily	HCl	+	Yes	
* $C_{(1)}9$	7/23/72	Emily	CO_2	+	Yes	
* $C_{(1)}10$	7/23/72	Emily	CO_2	+	Yes	
$C_{(1)}11$	7/23/72	Emily	CO_2	-	Yes	
$C_{(1)}12$	7/23/72	Emily	CO_2	-	Yes	
* $C_{(2)}8$	8/9/72	Emily	CO_2	+	Yes	
* D 2	6/22/74	Emily	CO_2	+	Yes	
D 11	6/22/74	Emily	HCl	+	Yes	
* E 2	7/16/74	Emily	CO_2	+	Yes	
* F 2	8/21/74	Emily	CO_2	+	Yes	1 X inoculum
* F 3	8/21/74	Emily	CO_2	+	Yes	1 X inoculum
* F 15	8/21/74	Emily	CO_2	+	Yes	1 X inoculum
* F 16	8/21/74	Emily	CO_2	+	Yes	1 X inoculum
* F 17	8/21/74	Emily	CO_2	+	Yes	1 X inoculum
* F 18	8/21/74	Emily	CO_2	+(a)	Yes	1 X inoculum
* F 19	8/21/74	Emily	CO_2	+(a)	No	1 X inoculum
* F 22	8/21/74	Emily	CO_2	+	Yes	1 X inoculum
* F 13	8/21/74	Emily	CO_2	+	Yes	5 X inoculum
* F 14	8/21/74	Emily	CO_2	+	Yes	20 X inoculum

(a) = CO_2 begun day 1; nutrients added days 6 - 10.

Table 4 - continued

SERIES & EXPERIMENT	DATE	LAKE	AGENT	NUTRIENTS	SHIFT	COMMENTS
* G 2	9/24/74	Emily	CO_2	+	Yes	
H	10/74	Emily	CO_2	+(b)	Yes	lab
H	10/74	Pleasant	CO_2	+(b)	Yes	lab
H	10/74	Powderhorn	CO_2	+	Yes	lab
H	10/74	Como	CO_2	+(b)	Yes	lab
H	10/74	Emily	CO_2	+	Yes	lab 1 X inoc.
H	10/74	Emily	CO_2	+	Yes	lab 2 X inoc.
H	10/74	Emily	CO_2	+	Yes	lab 4 X inoc.
I	10/74	Quarry	CO_2	+	Yes	lab
I	10/74	Quarry	CO_2	+	Yes	lab
I	10/74	Quarry	HCl	+	No	lab
I	10/74	Quarry	HCl	+	No	lab
$J_{(1)}^{1}$	7/28/75	Emily	CO_2	-	Partial	
$J_{(1)}^{2}$	7/28/75	Emily	CO_2	-(c)	No	
* $J_{(1)}^{4}$	7/28/75	Emily	CO_2	+	Yes	
* $J_{(1)}^{5}$	7/28/75	Emily	CO_2	+	Yes	
$J_{(1)}^{6}$	7/28/75	Emily	CO_2	+(c)	Partial	
* $J_{(1)}^{7}$	7/28/75	Emily	CO_2	+	Partial	
$J_{(2)}$	7/76	Ardmore	CO_2	+	Yes	lab
$J_{(2)}$	7/76	Lobe	CO_2	+	Yes	lab
$J_{(2)}$	7/76	Ryan	CO_2	+	Yes	lab
$J_{(2)}$	7/76	Half Moon	CO_2	+	Yes	lab
$J_{(2)}$	7/76	Dickey	CO_2	+	Yes	lab
$J_{(2)}$	7/76	Shady Oak N.	CO_2	+	Yes	lab

(b) = control also shifted.

(c) = CO_2 added only briefly at the beginning.

algae to compete under various conditions. However, it soon became obvious that the algal shift might have a practical use in lake restoration. It was also obvious immediately that one cannot lower the pH of a whole lake to 5.5 as a practical procedure, so the extent to which the shift would occur at higher pH values was of interest. Accordingly, several series of field experiments were done, all at Lake Emily.

The experimental setup was similar to that used in the pH 5.5 experiments. Control of pH was accomplished through addition of CO2 to one set of bags and HCl to a second set. The pH adjustments were accomplished as follows: pH electrodes, emerging through rubber stoppers in the ends of one foot long sections of one-inch diameter plastic tubing, were inserted several inches below the surface of each bag to be regulated. Each electrode was connected to a pH stat housed in a waterproof shelter on the central raft. Each pH stat, which was wired into a central multiple cam timer, controlled a stainless-steel solenoid valve. The valves were used to meter either compressed CO2, or HCl (10-30% depending on circumstances), into the bags. Running to the raft from a small shed on shore were a 110-volt A.C. power line (connected to a ground fault interrupter), a 1/4-inch polyethylene tube carrying compressed CO2 at 20 PSIG from cylinders in the shed, and a 1/2-inch compressed air line from a 1/2 HP electric compressor.

Every day at 10 a.m. and 2 p.m. a timer in the shed sent power to the timer on the raft. Two pH stats were thus activated to deliver either CO2 or HCl (from a pressurized glass bottle on the raft) to the 2 bags to which their solenoids and pH electrodes were attached, to adjust the pH levels down to the appropriate pre-set values. At the same time compressed air was released from tubes on the bottoms of the bags in sufficient volume to mix the water thoroughly so that the pH adjustment would be accurate. After four minutes all units shut off and after two more minutes the next pair of bags was circulated and adjusted. This cycle went on until all the bags were done, whereupon the whole system shut down until the next cycle. No pH adjustments were made at night.

Three such graded pH experiments were done in 1974: numbers D, E, and F, beginning on June 22, July 16, and August 21 respectively. As in the pH 5.5 experiments, N and P were added to all of the bags. The experimental conditions and results are shown in Table 5.

The results were best in experiment D, where the bags adjusted with CO2 to pH 5.5, 6.5, 7.5, and 8.5 all showed the shift, although it was incomplete at pH 7.5. The bags adjusted with HCl showed the shift at pH values of 5.5, 6.5, and 7.5. The bag adjusted to pH 8.5 with HCl retained its blue-green population, as did the control bag, #12, where the pH exceeded 9.5 as a result of photosynthesis.

In experiment E the bags adjusted to pH 6.5 and 7.5 with CO2 failed to shift and the pH 8.5 CO2 bag and pH 7.5 HCl bag showed partial shifts only. The control remained blue-green.

In experiment F both pH 5.5 bags shifted. Neither bag at pH 6.5 did so. At pH 7.5 one bag remained predominantly blue-green and the other showed a partial shift. Of the bags at pH 8.5 one did not shift and the other did. However, the former actually was at pH 7.5 and the latter at 6.5 because of poor control by the pH stats. The control remained blue-green.

Thus the results were not clear-cut and seem to show an element of chance. However, they do show that in nine out of sixteen cases the change occurred, completely or partially, at pH values above 5.5 and at values as high as 8.5. Thus the possibility of shifting algal populations from blue-greens to greens as a practical measure is reinforced.

TABLE 5

Experiments in which pH was maintained at predetermined values. N and P added in all cases.

SERIES & EXPERIMENT	DATE	pH	AGENT	SHIFT
D 2	6/22/74	5.5(a)	CO_2	Yes
D 4	6/22/74	6.5	CO_2	Yes
D 5	6/22/74	7.5	CO_2	Partial
D 6	6/22/74	8.5	CO_2	Yes
D 11	6/22/74	5.5(b)	HCl	Yes
D 7	6/22/74	6.5	HCl	Yes
D 8	6/22/74	7.5	HCl	Yes
D 9	6/22/74	8.5	HCl	No
D 12	6/22/74	>9.5(c)	-	No
E 2	7/16/74	5.5(a)	CO_2	Yes
E 3	7/16/74	6.5	CO_2	No
E 5	7/16/74	7.5	CO_2	No
E 7	7/16/74	8.5	CO_2	Partial
E 8	7/16/74	7.5	HCl	Partial
E 1	7/16/74	>9.5(c)	-	No
F 2	8/21/74	5.5(a)	CO_2	Yes (d)
F 3	8/21/74	5.5(a)	CO_2	Yes (d)
F 5	8/21/74	6.5 (7.0)	CO_2	No (d)
F 6	8/21/74	6.5 (7.0)	CO_2	No (d)
F 7	8/21/74	7.5	CO_2	No (d)
F 8	8/21/74	7.5	CO_2	Partly(d)
F 9	8/21/74	8.5 (7.5)	CO_2	No (d)
F 10	8/21/74	8.5 (6.5)	CO_2	Yes (d)
		>9.5(c)	-	No (d)

( ) = actual pH
(a) = excess CO_2, no stat
(b) = HCl, no stat
(c) = no stat, high pH from photosynthesis
(d) = 1 x inoculum

Biological Details of the Shift

At this point it is worth considering the nature of the shift in some detail. Table 6 is a listing of the algae predominating at the beginning of each series of experiments, and of the algae making up the bulk of the population after the shift had occurred. The point of greatest interest here is that filamentous and gelatinous blue-greens give way to mostly greens of a few restricted genera. The general effect is best portrayed by a photograph as in Figure 8. In the case shown (Expt. D2 Table 4) the shift occurred at a pH of 5.5 and resulted in mostly Chlorella and Scenedesmus. The same initial algal population was subjected to pH 8.5 (Expt. D6 Table 5) and again the resulting algae were mostly Chlorella and Scenedesmus. These changes at pH 8.5 are summarized in Figure 9.

Frequently there is more than one species of the dominant green genus. For example, in the pH 8.5 experiment described above, following the shift there were no fewer than 22 species and subspecies of Scenedesmus alone.

Rate of the Shift

The rate at which the shift occurs is variable. We believe that, in general, experiments done in the late spring and perhaps in the fall result in quicker shifts than do those done in mid-summer. This may be related to temperature, but it may also be because a larger inoculum of green algae is present earlier and later. In one case (Series F), suspecting too few green algae to begin with, we inoculated all of the bags with green algae (mostly Scenedesmus and Chlorella) that had resulted from a shift in the previous experiment. The normal inoculum used was a 2-gallon pail of the "greens" to one bag, or about 1 volume/125 volumes. To determine the effect of inoculum size we inoculated two other bags (F13 and F14) with 5x and 20x this quantity respectively. The bags with 1x and 5x inoculum appear to have undergone the shift after approximately 18 days treatment with CO2 and nutrients at pH 5.5, but the bag with 20x inoculum had shown a clear shift by day 13.

Obviously then, it is necessary to have greens present to grow. But not all of the greens do respond. On occasion some have not changed in abundance while others have even decreased in number.

In looking at the effects of pH on the rate of the shift the data are equivocal. In Experiment D (Table 5) where pH values of 5.5, 6.5, and 8.5 were achieved with CO2, the shift occurred in 11, 10, and 10 days respectively. When the pH was maintained at 5.5, 6.5, and 7.5, with HCl, the shift required 9, 10, and 13 days respectively -- suggesting, under these conditions, a slightly faster response at the low pH.

One fact about the shift is that it often appears to occur somewhat precipitously after a variable period with no apparent change. For example, we have on several occasions examined the bags on say, Friday evening, and decided that little change had occurred, while on the following Monday the algae seemed to be almost all Chlorella and Scenedesmus.

Resistant Algae

Of perhaps as much interest as what species of algae become abundant is the question, what species of blue-greens if any are resistant to the shift? As noted earlier, in 19 of 20 cases where nutrients were added and the pH was lowered to 5.5 with CO2, the shift occurred. However, in several instances where other pH levels were used, the shift did not occur. Does this mean that certain species of algae are resistant? Probably not. For example, in experiments D2 and D6 carried out with CO2 at pH 5.5 and 8.5 respectively, all of the following disappeared to the point where they were below enumeration levels: Oscillatoria, Anabaena, Chroococcus, Gomphosphaerium,

TABLE 6

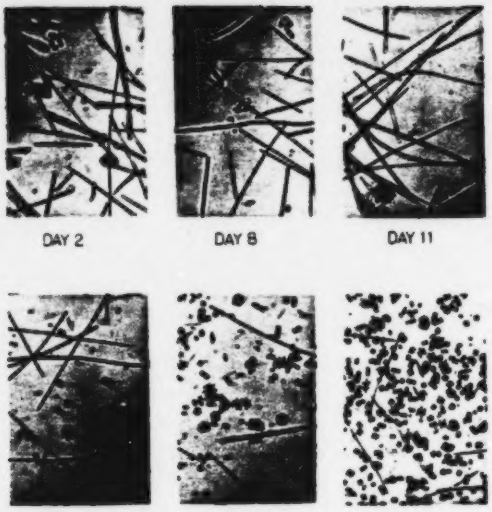
Algae predominating before and after the shift.
(* = shift done with chlorine instead of pH change)

Experiment Series	Algae before shift	Algae after shift
B	Oscillatoria Anabaena	Scenedesmus Chlorella
C	Microcystis Oscillatoria Anabaena Aphanizomenon	Chlorella Dictyosphaerium Scenedesmus Chlamydomonas
D	Oscillatoria Anabaena Gomphospheria	Chlorella Scenedesmus Coelastrum
E	Oscillatoria Aphanizomenon	Scenedesmus Chlorella
F	Oscillatoria	Scenedesmus Stichococcus Chlorella
H Emily	Oscillatoria Scenedesmus	Scenedesmus Chlorella
H* Emily	Oscillatoria Scenedesmus	Chlorococcus Scenedesmus
H Pleasant	Oscillatoria Ankistrodesmus	Chlorella Oscillatoria
H* Pleasant	Oscillatoria Ankistrodemus	Mougeotia Scenedesmus
H Powderhorn	Oscillatoria Scenedesmus	Scenedesmus Chlorella
H Como	Oscillatoria Scenedesmus	Scenedesmus Chlorella
H* Como	Oscillatoria Scenedesmus	Nitzschia Chlorococcus
J (1)	Chroococcus Microcystis Anabaena	Cosmarium Selenastrum Dictyospherium Gleocystis Cryptomonas
J* (1)	Chroococcus Microcystis Anabaena	Ankistrodesmus Selenastrum Scenedesmus

Table 6 - continued

Experiment Series	Algae before shift	Algae after shift
$^{J}(2)$ Ardmore	Aphanizomenon Oscillatoria	Closteriopsis Chlorella
$^{J}(2)$ Lobe	Merismopedia Anabaena	Chlorella Ankistrodesmus
$^{J}(2)$ Ryan	Gleocystis Anabaena	Chlorella Ankistrodesmus
$^{J}(2)$ Half Moon	Tribonema Microcystis	Ankistrodesmus Nitzschia
$^{J}(2)$ Dickey	Oscillatoria Chroococcus	Closteriopsis Selenastrum
$^{J}(2)$ Shady Oak N.	Oscillatoria Chroococcus	Scenedesmus Sphaerocystis

In certain of the experiments Phormidium may have been present with Oscillatoria. We have used the name Oscillatoria alone.



DAY 2 DAY 8 DAY 11

DAY 14 DAY16 DAY 19

Fig. 8. Photographs showing the shift induced by addition of N & P and CO_2 to a pH of 5.5 (Expt. D2, Table 4).

and *Aphanizomenon*. Thus, these species were vulnerable at both pH levels. However, examination revealed that *Oscillatoria* was beginning to reappear after a week at pH 5.5 but remained absent at pH 8.5. Apparently, as with most organisms, some individuals are more resistant than their fellows and, if in a particular experiment the shift does not occur, it probably is for some reason other than that the algae are resistant species.

Reversibility of the Shift

Several attempts were made to determine if the shift was reversible, i.e. if once again raising the pH or allowing it to increase by itself through photosynthesis would bring back the blue-green dominance. One such attempt was experiment C1(6) This experiment was begun on 7/27/72 with the algal population consisting of *Microcystis*, *Oscillatoria*, *Anabaena*, and *Aphanizomenon*. The enclosure was treated with N and P and HCl at pH 5.5 and by 8/3 *Scenedesmus* and *Dictyosphaerium* were abundant. By 8/8 *Scenedesmus* was predominant. On 8/8 the pH was raised to 9.2 with KOH and more N and P were added. On 8/16 *Scenedesmus* was still the dominant alga but by 9/24 *Anabaena*, *Oscillatoria*, and *Aphanizomenon* had regained dominance.

Perhaps the best such experiment from the technical point of view was that begun on August 21, 1974. The contents of bag E2, which had been treated with CO2 and N and P at a pH of 5.5, had become predominantly green (*Scenedesmus*), between 7/16 and 8/8. From 8/8 to 8/21 the bag was allowed to sit with no further additions of CO2. It remained green. On 8/21 it was divided into two bags. One had CO2 and N and P added as originally. The other had its pH raised to 9.5 with KOH. The first retained its dominance of *Scenedesmus* until the experiment was ended on 9/18, although a few filaments of *Oscillatoria* did remain. The second bag, with the high pH, behaved similarly at first but began to develop more and more filamentous blue-greens. By 9/19 the population, although it still had many *Scenedesmus*, was once again rich in filamentous blue-greens.

In another example, bag C10, which had been shifted to greens at pH 5.5 with CO2 and N and P, had its pH raised with KOH but, by chance, only to 6.7. Some reversion did occur but the population remained about half greens and half filamentous blue-greens.

Finally, one case exists in which reversion occurred without addition of KOH. This was experiment C9. Treatment begun on 7/23/72 caused the blue-greens to shift over to *Chlorella* and *Scenedesmus* and by 8/16 a notation was made that the bag contained "all greens, no blue-greens". On 8/25 the CO2 was shut off. By 9/24 *Scenedesmus* was still abundant but *Chroococcus* and many filamentous blue-greens were present. The pH had not been monitored but undoubtedly it must have risen as a result of photosynthesis.

Mechanism of the Shift

The reason for the shift is still not known. One might presume that lowering the pH to 5.5 with CO2 or HCl would be, as suggested by Brock (1973), detrimental to the blue-green algae and in fact might kill them. However, notwithstanding this possibility, we have been able to culture blue-greens, including *Oscillatoria*, successfully at pH 5.5. Furthermore, the shift has been made to occur at pH values as high as 8.5, and surely this cannot be claimed to be a physiologically damaging pH for blue-greens. The fact that the shift occurs only sometimes at a pH value above 5.5 also suggests the view that it is not the pH *per se* that is important.

Originally we believed that the shift was due to the superior CO2 uptake kinetics of the blue-green algae, and that reversal of competition to favor the greens was brought about by supplying CO2 either directly by bubbling it in, or by lowering the pH so as to decompose the native alkalinity of the lake waters. Although Long (1976) recently has confirmed this difference in CO2 uptake ability, we do not believe that this is the sole explanation. That is, the change does not appear to be one in which green algae slowly replace blue-greens. In most cases where the shift has occurred there has been a rapid decrease of the blue-greens -- as indicated by changes in numbers and decreases in chlorophyll -- before the greens have begun growing rapidly. The blue-green decrease and the green increase thus appear to be separate phenomena. Furthermore, phosphorus and perhaps nitrogen appear to be involved. When the blue-greens decrease high concentrations of PO4-P and NH3-N are found in the water. These substances are not left over from the initial addition that is usually made, but apparently have been liberated from the blue-green cells. That is, in control systems where the pH remains high and no shift occurs, phosphate does not appear in solution. However, where the shift occurs, phosphate and ammonia do show up (Fig. 10). Thus, of 42 examples where the data are available, in 17 there was a positive correspondence between the shift and the release of phosphate. In 18 there was a positive correspondence between no shift and no release of phosphate; and in the remaining 7 cases there was neither correspondence. However, in 4 of the last 7 the release began but the experiment was terminated, and in these the shift might have taken place if given time.

Thus the release of nutrients from the blue-greens apparently must occur before the greens grow. The nutrients then are, of course, used by the greens. Curiously enough, the addition of nutrients at the beginning of the experiment, even when greens are rare, facilitates the shift. If N and P are not added, the shift, even at pH 5.5, is slow and partial. But if N and P are added, even though they are absorbed rapidly by the blue-greens, the eventual shift is more dramatic.

In one series of experiments the CO2 and nutrients were added beginning at different times, e.g. CO2 from the beginning, nutrients at 6-10 days, or nutrients at the beginning and CO2 from the sixth day, etc. In most of these cases the shift occurred. However, in one case (F19) where the N and P were added over the period 6-10 days while the CO2 was begun on day 1, the shift did not occur, although in the duplicate (F18) it did.

Experiment J(1) included two bags in which CO2 treatment was done for only about one half hour at the beginning of the experiment. Bag J(1) 2 was treated in this way but with no N and P and failed to shift. Bag J(1) 6, which had N and P added showed a partial shift.

The CO2 also acted as a nutrient in the sense that generally more algae developed when CO2 was used to lower the pH than when HCl was used. But this is understandable as at the high concentrations of phosphorus (about 200 micrograms/l) and nitrogen (1000 micrograms/l), CO2 could be limiting, particularly in a system isolated from the rest of the lake.

One hypothesis about what might be happening to cause the shift is that the blue-greens are being destroyed by cyanophage or bacteria as a result of the pH manipulations (Lindmark, 1979, Lindmark and Shapiro, in prep.), and that once the blue-greens are gone th the few greens initially present no longer have competition for the nutrients. Furthermore, green algae generally require a higher concentration of phosphate for rapid uptake (Shapiro, unpubl.) and now have it available to them. The reason that nutrients are required may be to stimulate the blue-greens to active growth in which condition they may be more susceptible to the destructive agent.

This hypothesis is supported by certain observations. For example, when Oscillatoria disappears, the filaments do break up first as though certain cells were being lysed. Also not all blue-greens are affected. Thus,

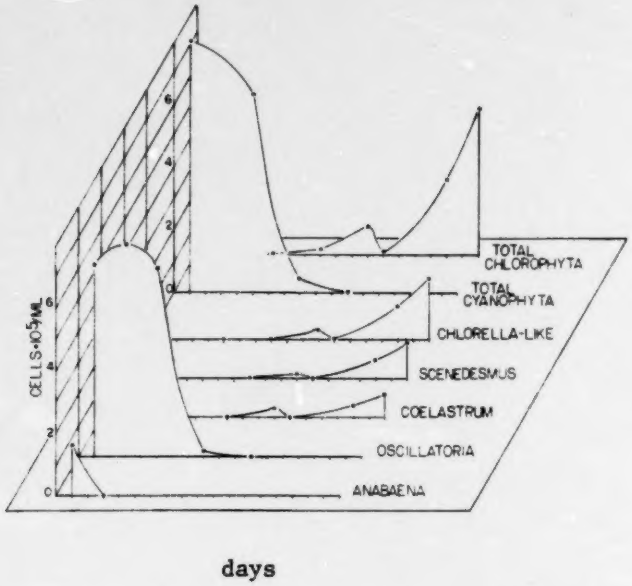


Fig. 9. Changes in the algal population brought about by adding N & P and maintaining a pH of 8.5 with CO_2. (Expt. D6, Table 5).

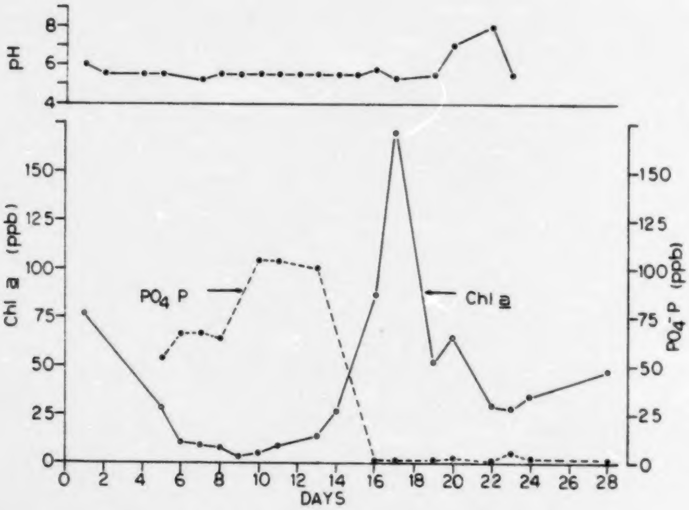


Fig. 10. Changes in chlorophyll and the release of phosphate at pH 5.5. (Expt. D2, Table 4).

Raphidiopsis, for example, may continue to grow and increase in numbers even though Oscillatoria is decreasing in numbers. Also, even when Oscillatoria has decreased it sometimes regrows in the same system, e.g. D2, indicating that it is not the pH, which remains low, that is the important feature.

An observation that bears on the mechanism of the shift concerns the effect of arsenic on the phenomenon. In earlier work (Shapiro, unpubl.) it has been shown that whereas blue-green algae distinguish between phosphate and arsenate, taking up the former and leaving the latter, green algae do not make this distinction and arsenate slows their uptake of phosphate. Accordingly, we reasoned, if phosphate transfer from lysed blue-greens to greens is important, would the addition of arsenate slow or stop the shift? Two experiments were done to test this. The first was begun on 7/16/74. Two bags were set up at Lake Emily, both treated with CO2 to pH 5.5 and with N and P added as usual. However, at the beginning of the experiment 100 micrograms/liter of arsenic as sodium arsenate was added to one of the bags, with the other serving as a control. Both bags behaved similarly until about the fourteenth day, i.e. chlorophyll fell to a low level by day 10 and PO4-P was found in solution as expected. However, the control bag shifted to greens with a maximum of chlorophyll by day 17, while the "arsenate" bag did not reach its peak of green algae until day 24. Thus the presence of the arsenate caused a one-week delay although the shift did occur and the final chlorophyll levels reached were similar.

The second experiment, begun on 8/21/74, was conducted in the same way as the first. Again, the control and experimental bags behaved similarly for the first two weeks. However, the control bag shifted shortly thereafter with a significant increase in chlorophyll (to about 90 micrograms/liter) while no shift took place in the presence of the arsenate, and the chlorophyll reached only 32 micrograms/liter. Thus, in this case the arsenate completely prevented the shift from occurring.

Finally, in considering the mechanism of the shift, the effects of chlorine must be taken into account. It had previously been shown that chlorine limited the rate of phosphate uptake by blue-green algae more than by green algae (Shapiro, unpubl.). Therefore it was reasoned, if phosphate is added to a mixture of algae in the presence of chlorine, the green algae should have an advantage and the shift from blue-greens to greens might occur even at a high pH.

In the first such experiment, begun 7/16/74, 750 micrograms/l of chlorine were added to the experimental bag along with N and P while the control bag had only N and P added. The two bags behaved similarly in that both developed crops of algae containing approximately 300 micrograms/l of chlorophyll. No shift occurred in either one although phosphate was released and later reabsorbed in the chlorine bag. Both had pH values of 10.

The second chlorine experiment was begun on 8/21/74 and was done in duplicate. In both cases the blue-greens were greatly diminished in the presence of chlorine and there was a shift to greens even though the pH was between 10 and 11. As in the previous experiment PO4 was released in both experimental bags. Another field experiment in Lake Emily in July 1975 and 4 of 5 laboratory experiments on other lakes were all successful in that chlorine caused the shift at high pH.

Thus, these experiments along with the arsenate work support the idea that the phosphate uptake by greens and the subsequent growth of the greens is a phenomenon separate from the demise of the blue-greens. However, the reason for the blue-green decline still remains unknown. Recently we have begun using autoradiography with C14 and P32to help elucidate the mechanism.

APPLICATIONS

As noted earlier the work described has both theoretical and practical significance. From the theoretical standpoint the work may support the idea of better CO2 uptake and use kinetics in blue-green algae. It certainly appears to support the notion that if phosphate becomes available those algae that are able to out-compete others for it will become predominant. From the practical standpoint, the work may have great value in lake restoration. As described in a previous paper (Shapiro *et al*., 1975) we foresee the day when it will be possible to manipulate small or moderate-sized lakes to improve them. Part of the manipulation may involve converting the population of algae from forms inedible by zooplankton to forms that can be eaten by them. In other words, we consider it possible to bring about a blue-green to green shift in whole lakes. The relationship of our studies to such shifts brought about by artificial circulation has already been described (Shapiro *et al*., 1975) and our continuing studies indicate that the same factors operating to cause the shifts in the work reported herein are responsible for shifts in such circulation ventures.

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II.B. THE EFFECTS OF ARTIFICIAL CIRCULATION ON ALGAL POPULATIONS*

INTRODUCTION

Artificial circulation has often been proposed as a method for controlling algal blooms in lakes and reservoirs. However, the response of the phytoplankton to destratification has been quite variable. A number of investigators have noted changes in species composition, and a shift from predominantly blue-green algae to green species is often reported (Symons et al. 1969; Malueg et al. 1971; Haynes 1971, 1973, 1975; Sirenko et al. 1972; Weiss and Breedlove 1973). However, in other cases, blue-green species have increased following circulation attempts (Ridley et al. 1966; Lackey 1973; Knoppert et al. 1970). Certain species such as Oscillatoria rubescens (Bernhardt 1967; Weiss and Breedlove 1973) and Mallomonas (Lackey 1973) appear to be particularly sensitive to destratification and are quickly eliminated. Changes in total algal biomass have also been variable, with some authors reporting an increase (Hooper et al. 1952; Ridley et al. 1966; Knoppert et al. 1970; Drury et al. 1975), and others a decrease (Bernhardt 1967; Robinson et al. 1969; Malueg et al. 1971) in algal biomass following destratification. It is clear, then, that whole lake circulation can produce a variety of responses in the phytoplankton, not all of which are beneficial, and if circulation techniques are to be used effectively we must first understand the mechanisms underlying these responses. The primary objective of our research has been to investigate these response mechanisms. We recognized that these mechanisms would probably involve complex interactions among many physical, chemical and biological factors, and that the response of the algal community could, therefore, only be understood within the context of a larger theoretical framework consistent with both the destratification experience of others and our knowledge of the structure and function of aquatic ecosystems. Accordingly, we began our investigations by constructing such a framework. Potential response mechanisms were then identified and evaluated in controlled field experiments.

THEORETICAL FRAMEWORK

Effects of Destratification on Species Composition

Changes in algal species composition usually reflect the differential effects of various growth factors. A number of these factors can change as a direct consequence of artificial circulation.

During circulation hypolimnetic water, rich in CO_2 and nutrients, is introduced into the euphotic zone. If CO_2 is introduced faster than it is consumed during primary production or vented to the atmosphere it will accumulate, causing a shift in the carbonate equilibria and a corresponding drop in pH. The magnitude of this pH drop will depend primarily on the amount of CO_2 present in the hypolimnion of a lake and on the buffering capacity of the water. Several investigators have reported a drop in pH during artificial circulation and in many cases this change was accompanied by a shift in the algae from blue-green to green species (Symons et al. 1969; Haynes 1971, 1973, 1975; Malueg et al. 1971; Weiss and Breedlove 1973). It is also possible for the pH to increase or remain high following destratification if autotrophic consumption of CO_2 exceeds the rate of introduction via mixing. Knoppert and associates (1970) reported very high pH levels and an increase in the abundance of blue-green algae following destratification. There is considerable support for the idea that blue-greens have a competitive advantages over green algae when the pH is high and that the advantage shifts in favor of the green algae in a low pH environment. King (1970) found that algal communities in sewage lagoons were dominated by blue-greens when the pH was high and by green algae when the pH was low. Shapiro (1973) demonstrated that the composition of natural phytoplankton communities could be shifted from predominantly blue-green algae to green algae by the direct addition of CO2. However, it is not clear whether these shifts are a result of competition for CO2 as suggested by King (1970) or the result of some other phenomenon. For instance, Lindmark (1979) has shown that low pH can stimulate cyanophage activity, giving non-bluegreen species a selective advantage. Hypolimnetic water introduced into the euphotic zone during circulation can also contain high concentrations of Si, N, P, and other growth factors. Several investigators have noted an increase in the surface concentration of Si after the onset of mixing (Ridley *et al*. 1966; Hedman and Tyley 1967).

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*by Bruce Forsberg and Joseph Shapiro

This increased availability of Si may offer a selective advantage to diatoms which have a unique (Ridley *et al*. 1966; Hedman and Tyley 1967) requirement for this mineral. An increase in the silica concentration during the mixing of one British reservoir was followed by a bloom of the diatom *Asterionella formosa* (Anon. 1965-66).

Artificial destratification is often accompanied by an increase in euphotic zone concentrations of nitrogen (Brezonik *et al*., 1969, Haynes 1971); and phosphorus (Hooper *et al*. 1952, Wirth and Dunst 1967, Slack and Ehrlich 1967, Leach *et al*. 1970, Weiss and Breedlove 1973). However, some investigators have reported a decrease in TP during mixing (Haynes 1975, Drury *et al*. 1975, Weiss and Breedlove 1973) presumably due to chemical co-precipitation with iron (Lee 1970). The response of each algal species to changes in the availability of phosphorus and nitrogen will depend on its own physiological requirements as well as competitive interactions with other species.

Concentrations of Fe, Mn, B, Al, Cu, and H2S may also change following destratification (Toetz *et al*. 1972) but the effect of these substances on algal growth is not well understood.

Changes in water temperature during destratification can also influence species composition. Surface temperatures generally decrease after the onset of mixing (Toetz *et al*. 1972). The rates of many physiological processes such as photosynthesis should decline with temperature and any differences between species in these temperature effects will tend to produce changes in species composition.

Artificial circulation can also influence the light climate of the algae by decreasing the proportion of the population which resides in the euphotic zone thereby reducing the relative availability of light. Assuming that the algae are uniformly distributed in the mixed layer, the proportion of the population residing in the euphotic zone is equivalent to the ratio of the depth of the euphotic zone to the depth of the mixed layer, Ze/Zm. This ratio can be further described, according to the Lambert Bouger Law, by the equation:

$$\frac{Ze}{Zm} = \frac{\ln(Io/Ize)}{(aE_c)/Zm + Ew} \cdot \frac{1}{Zm} = \frac{4.6}{aEc + Ew\ Zm} \qquad (1)$$

where, Io = surface irradiance
Ize = irradiance at the depth of the euphotic zone = 1%
Ec = partial extinction coefficient for chlorophyll a (m2 mg Chl a-1)
Ew = residual extinction coefficient of the water (m-2)
a = areal standing crop of chlorophyll a (mg Chl a m-2)
Ze = depth of the euphotic zone in meters
Zm = depth of the mixed layer in meters.

Equation 1 indicates that as the mixed depth is increased both the depth of the euphotic zone and Zm will increase. However, the net result will always be a reduction in the ratio Ze/Zm. Figure 11 indicates the change in Ze/Zm expected as the mixed depth is increased, assuming constant values for a, Ew and Ec. It is clear the Ze/Zm will decrease as a continuous function of Zm and that the most significant decrease in light availability will occur at shallow mixed depths. This reduction in relative light availability following destratification should result in lower integral rates of photosynthesis, π (mg C m-2 d-1). Although this is not always observed, Haynes (1973) reported lower integral rates of photosynthesis following destratification in Kezar Lake, New Hampshire. The importance of this change to the phytoplankton depends on the relative magnitude of algal loss factors (e.g. respiration, excretion, sinking and grazing). If Zm is increased to the point where π equals the total integral loss rate, L, then growth will cease. This mixed depth was defined by Sverdrup (1953) as the critical depth. When the mixed depth exceeds the critical depth the population will decline. The response of each population to changes in light availability, then, will be expressed as a change in the ratio, π /L. If light is the primary factor limiting photosynthesis and L remains constant, π /L should decrease following destratification and the response should be different for each species. This differential effect of light limitation should influence species composition during mixing.

Two additional factors which can change as a consequence of artificial circulation and influence species composition are the loss rates due to sinking and grazing. In the absence of turbulence, the sinking loss rate for a population is equal to the terminal sinking velocity divided by the mixed depth (Bella 1970, Bannister 1974b). As the mixed depth is increased then the sinking loss rate should decline. In addition, the increased turbulence resulting from artificial destratification should also reduce the sinking velocity and the net effect of decreasing the sinking velocity and increasing the mixed depth should be to greatly reduce the sinking loss rate. This effect should benefit heavier species, such as diatoms, the most and may result in an increase in their relative abundance.

Environmental variables affecting herbivorous zooplankton can also change dramatically after destratification. In lakes with anoxic hypolimnia zooplankton are normally restricted to the strata above the thermocline. After destratification, when bottom waters are no longer anoxic, zooplankton have been observed to increase their depth distribution (Brown et al. 1971, Fast 1971, Brynildson and Serns 1977). In addition to increasing the volume of zooplankton habitat, circulation may also reduce the adverse effects of predation by creating a dark refuge from visual predators and also by increasing the distance between predator and prey. These changes should lead to an increase in zooplankton abundance with larger organisms such as Daphnia, showing the greatest response. Several investigators have reported an increase in zooplankton abundance following destratification (Riddick 1957; McNall 1971; Brown et al. 1971; Brynildson and Serns 1977, Shapiro and

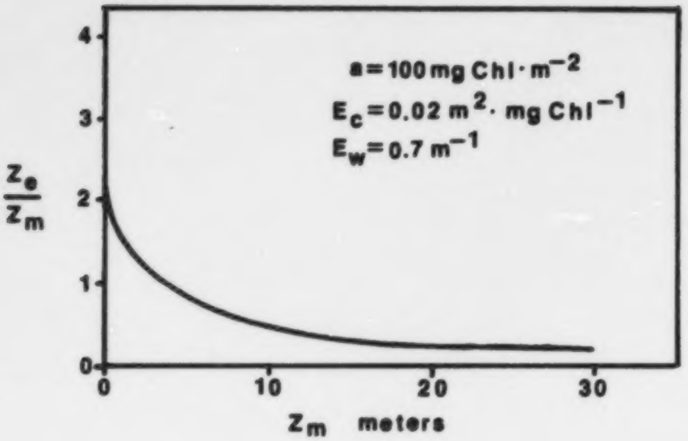


Fig. 11. Theoretical relationship between the ratio euphotic depth/mixed depth, and the mixed depth, assuming constant values for Ec, Ew and aerial standing crop. The figure indicates an increase in light limitation with increasing mixed depth.

Pfannkuch 1973). The increase in grazing pressure accompanying this change can have a great effect on the algal community.

Each of the factors described above can influence species composition and each can change as a direct consequence of destratification. Many of these factors are interrelated and all can act at the same time. We have tried to construct a theoretical framework which reflects the complexity of these interactions. Figures 12a and 12b summarize what we feel are the most important mechanisms through which whole lake circulation can influence species composition. We have tried to emphasize in these figures how the final result can depend on the mixing rate.

Effects of Destratification on Algal Community Standing Crop

The response of the entire algal community to artificial destratification is defined here as the change in algal standing crop summed for all species. Algal standing crop is equivalent to algal biomass and can be expressed on either a volumetric (e.g. mg Chl a m-3) or an areal (e.g. mg Chl a m-2) basis.

Bannister (1974b) has shown that the growth of a phytoplankton population will always be a complex function of many factors, operating simultaneously, and that the effect of a change in any one factor can only be understood within the context of a larger theoretical framework which considers all of them. A number of authors have developed mathematical models of algal growth in order to provide such a framework (Bella 1970, Lorenzen and Mitchell 1973, Megard and Smith 1974, Bannister 1974a, b, Lehman et al. 1975, Oskam 1978, and see Patten 1968 for a review of pre-1965 models) and, of these a few (Bella 1970, Lorenzen and Mitchell 1973, Oskam 1978) were developed specifically to describe the response of algal communities during artificial circulation. While we also recognized the need for a broad theoretical framework, we found the latter models lacking in several important respects. First, these models are all difficult to evaluate in the field and often require separate laboratory experiments to determine some model parameters. Second, they usually ignore algal losses due to sinking and grazing (except Bella 1970) which can be quite important in the community response. Finally, they either totally ignore (Bella, Oskam) or give only a crude treatment (Lorenzen and Mitchell) of the effects of nutrient limitation. In particular, nutrient limitation was described by a separate empirical yield term in the Lorenzen and Mitchell model which made it impossible to consider the effects of nutrient and light limitation simultaneously or to determine the effects of changes in nutrient concentration which might occur during mixing. In an attempt to remedy these deficiencies we have developed a new model of algal growth which has the following features:

1. All model parameters can be easily evaluated in the field.
2. All loss factors are considered including respiration, excretion, sinking, and grazing.
3. The effects of nutrient and light limitation are considered simultaneously.
4. The effect of changes in total nutrient concentrations is considered directly.

In essence, the model treats the algal community as a carbon pool, with carbon entering the pool through phytosynthesis and leaving through respiration, excretion, sinking and grazing. Algal growth then is described as the net result of both production and loss processes.

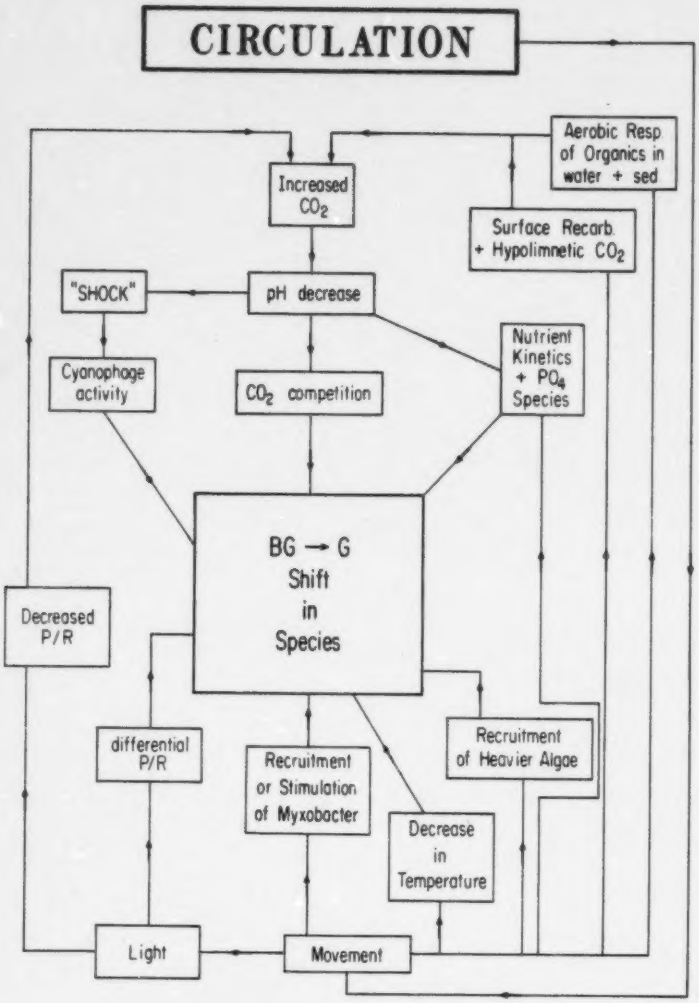

CIRCULATION
Aerobic Resp of Organics in water + sed
Increased CO2
Surface Recarb. + Hypolimnetic CO2
"SHOCK"
pH decrease
Nutrient Kinetics + PO4 Species
Cyanophage activity
CO2 competition
BG → G Shift in Species
Decreased P/R
differential P/R
Recruitment or Stimulation of Myxobacter
Recruitment of Heavier Algae
Decrease in Temperature
Light
Movement

Fig. 12 a. A diagrammatic representation of some of the results of whole-lake circulation and their role in causing a shift from blue-green algae to greens and diatoms.

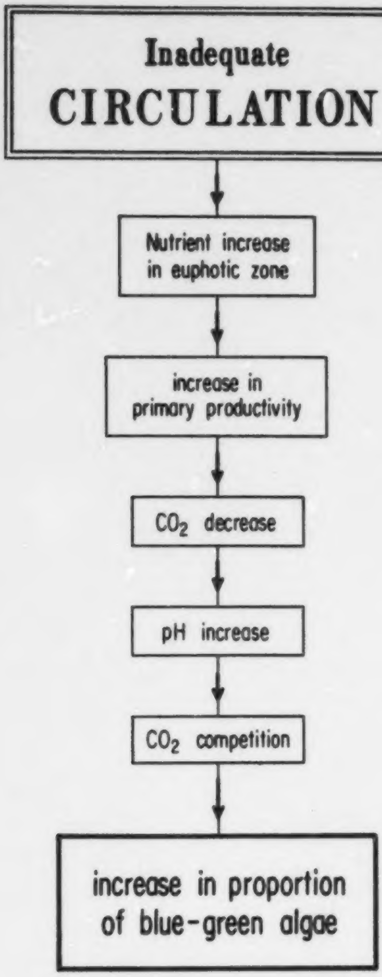

Inadequate CIRCULATION
Nutrient increase in euphotic zone
increase in primary productivity
CO_2 decrease
pH increase
CO_2 competition
increase in proportion of blue-green algae

Fig. 12 b. The probable manner by which inadequate circulation changes the proportion of algal types.

$$R = B - D \tag{2}$$

where, R = carbon specific growth rate (d-1)
B = carbon specific production rate (day-1)
D = specific loss rate (day-1)

The value of B depends on several additional factors including the availability of light and nutrients. The effects of these factors will be considered below. The daily integral rate of photosynthesis π (mg Cm-2 d-1) can be set equal to the area of a rectangle (Fig. 13) with one side equal to the value of the maximum daily volumetric rate of photosynthesis (p-max; mg C m-3 d-1) and the other side equal to the depth z' (Talling 1957a,b).

$$\pi = \text{p-max } z' \tag{4}$$

The parameter p-max can be redefined as the product of the maximum daily specific rate of photosynthesis (P-max; mg C mg Chl-1 d-1) and the concentration of chlorophyll a in the layer (c; mg Chl m-3).

$$\pi = \text{P-max } c\, z' \tag{5}$$

The parameter z' is derived empirically and has been found to depend (Talling 1957a,b) on the intensity of photosynthetically active irradiance at the surface (Io), the intensity of active irradiance at the depth z' (Iz'), the extinction coefficient of chlorophyll a (Ec; m2 mg Chl-1), the concentration of chlorophyll a, c, and the residual extinction coefficient of the water (Ew; m-1) such that:

$$Z' = \frac{\ln (Io/Iz')}{cEc + Ew} \tag{6}$$

The nutrient dependence of light saturated rates of photosynthesis was recently recognized by Senft (1978). He proposed a hyperbolic function similar to that of Droop (1974) to describe the relationship between light saturated rates and cellular nutrient quotas in laboratory cultures. Similarly, Forsberg (1980) has found that the value of P-max obtained from in situ incubations with natural populations depends on the ratio of the concentration of total phosphorus to chlorophyll (TP/c) according to the equation:

$$\text{P-max} = \text{P-}^{\text{sat}}_{\text{max}} \left(1 - \frac{Kq}{TP/c}\right) \tag{7}$$

where, $\text{P-}^{\text{sat}}_{\text{max}}$ = the maximum daily specific rate of photosynthesis under conditions of saturating nutrients (mg C mg Chl-1 d-1)
kq = the subsistence quota of TP or the ratio, TP/c, at which the value of P-max is zero (mg TP mg Chl-1).

After substitution equation 5 yields:

$$\pi = \left[\frac{\ln(Io/Iz')c\ \text{P-}^{\text{sat}}_{\text{max}}}{cEc + Ew}\right]\left[1 - \frac{Kq}{TP/c}\right] \tag{8}$$

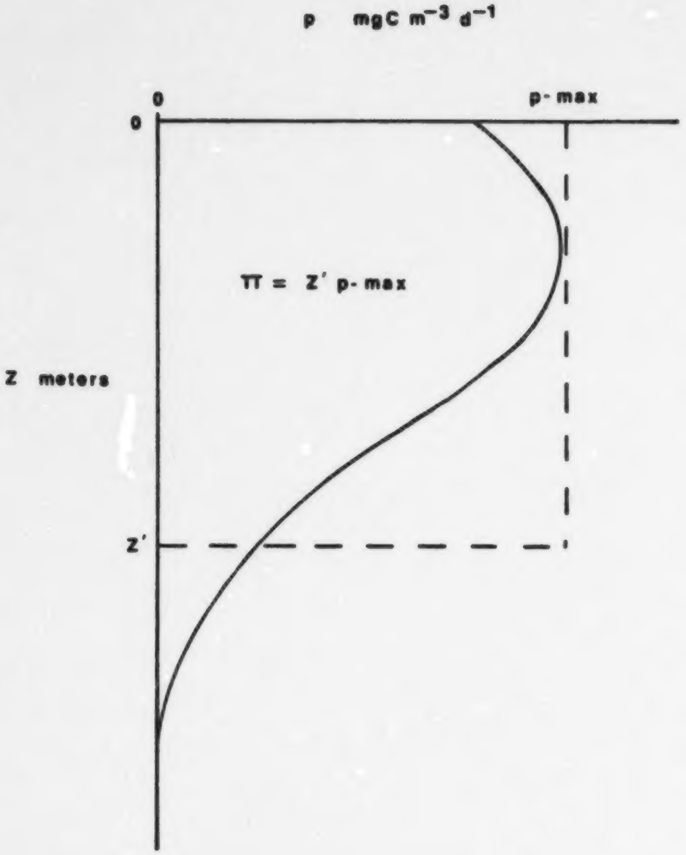

p mgC m⁻³ d⁻¹
0
p-max
0
ΤΤ = Z′ p-max
Z meters
Z′

Fig. 13. A typical photosynthesis-depth profile showing the equivalence between the depth integrated rate π (mgC m^{-2} d^{-1}) and the product p-max Z'.

B is then determined by dividing π by the areal standing crop of carbon (ΘZm C; mg C m-2).

$$B = \left[\frac{\ln(Io/Iz')c\ P\text{-}^{sat}_{max}}{cEc + Ew}\right]\left[1 - \frac{Kq}{TP/c}\right]\left[\frac{1}{Zm\Theta c}\right] \qquad (9)$$

where, Θ = the carbon:chlorophyll ratio in the phytoplankton (mg C mg Chl-1)
Zm = depth of the mixed layer (m).

The complete growth model is given by:

$$R = \left[\frac{\ln(Io/Iz')}{cEc + Ew}\right]\left[1 - \frac{Kqc}{TP}\right]\left[\frac{1}{Zm\Theta c}\right] - D \qquad (10)$$

This model is similar to that presented by Megard and Smith (1974). The only significant difference is our inclusion of the nutrient dependence terms which modifies the value of $P\text{-}^{sat}_{max}$. As pointed out by Megard and associates (1979) this model requires that the algae are uniformly distributed in the mixed layer and that saturating light intensities are achieved below the surface of the water column. The upper limit to algal standing crop can be determined by setting equation 10 equal to zero and solving for the equilibrium concentration of chlorophyll, c*.

$$C^* = \frac{\ln(Io/Iz')\ P^{sat}_{max} - D\Theta ZmEw}{EcD\Theta Zm + (\ln(Io/Iz')\ P\text{-}^{sat}_{max}\ Kq)/TP} \qquad (11)$$

This equation shows explicitly how the peak concentration of chlorophyll a depends on TP, Zm, algal loss factors, several light limitation parameters and Θ.

The equation can be used to predict how a change in any of these factors following destratification will influence algal standing crop. An increase in Zm, after destratification, will act to decrease standing crop while an increase in TP, which often accompanies destratification, will act to increase standing crop. However, the actual change in standing crop will depend on the combined action of all of the factors in equation 9. A number of these factors are sensitive to changes in algal species composition which might occur following destratification. For example, circulation may result in an increase in the abundance of blue-green algae (Fig. 12a). Since blue-green algae generally have lower sinking rates and are considered less edible than other groups, the loss rate, D, should decrease. This in turn would increase the yield of chlorophyll by increasing C*. Alternatively, mixing could result in a shift to green algae and diatoms. Since these species tend to be more easily grazed (Porter 1973) and have the highest sinking rates (Smayda 1970) this shift would lead to an increase in D and a decrease in C*. Changes in species composition may also influence the values of kc, Pmax, kq, and even ln (Io/Iz') since these characteristics can vary between species. Unfortunately we do not know enough about the variability in these latter parameters between species to predict how changes in species composition might affect their values at the community level. Several other factors can indirectly influence the levels of parameters in equation 10 during mixing. The survival and feeding rates of zooplankton may increase if the pH is lowered during mixing (Ivanova and Kiekowski 1972; Kring and O'Brien 1976) and this might result in a higher grazing rate and larger value of D. In addition, the TP concentration may decline if the mixing rate is sufficient to produce oxidizing conditions in the water column, since these conditions might cause phosphorus to co-precipitate with iron (Wetzel 1975) and result in an oxidized microzone at the sediment surface which would prevent the release of phosphorus from the sediments (Lee 1970). A change in surface temperature during mixing may also have significant effects on both P-max (Talling 1957a,b) and zooplankton survival (D). In summary, Figures 14a and 14b provide a diagrammatic representation of the mechanisms through which whole-lake circulation might influence the level of algal community standing crop. Again in these figures we have emphasized the potential importance of the mixing rate to the community response.

METHODS

General Design

The mechanisms proposed above were investigated in controlled field experiments. Eight experiments were carried out over a period of three years in two Minnesota lakes. Little Lake Johanna, a small (7.3 ha.) hyper-eutrophic lake with a maximum depth of 13 meters, was the site of experiments 1, 2, 4, 5, and 7. The remaining experiments (3, 6, and 8) were carried out on Twin Lake, a slightly larger (15 ha.) eutrophic lake with a maximum depth of 12 meters. Additional details on the limnology of Twin Lake can be found in Allott (1978). In each experiment 7-16 polyethylene cylinders were suspended from outriggers attached to rafts. A diagram of the general apparatus is shown in Figure 15. The rafts were always anchored in at least 9 m of water. All enclosures were made of 6 mil extruded clear polyethylene cylinders with a diameter of 1 meter and a depth of 8 meters. Each was open at the top, reinforced with PVC tubing on the sidewalls and either open or closed at the bottom. Open bottom enclosures were used to simulate natural lake conditions during artificial circulation. These enclosures were held open at the bottom by weighted (sand-filled) PVC hoops and lowered slowly from the surface to entrain an undisturbed water column. Closed bottom enclosures were used to study the effects of selected hypolimnetic constituents which are brought to the surface during artificial circulation. These enclosures were first filled with surface water, sunk to position, and then allowed to stratify thermally by conduction with their surroundings. (The thermocline was generally at a depth of 3 meters in Little Lake Johanna and 5 meters in Twin Lake during the experiments.) Once stratified, water was withdrawn from the hypolimnetic portion of an enclosure and after a variety of additions the water was then returned to the artificial hypolimnion. All additions were completed before mixing began and included various combinations and concentrations of the following substances.

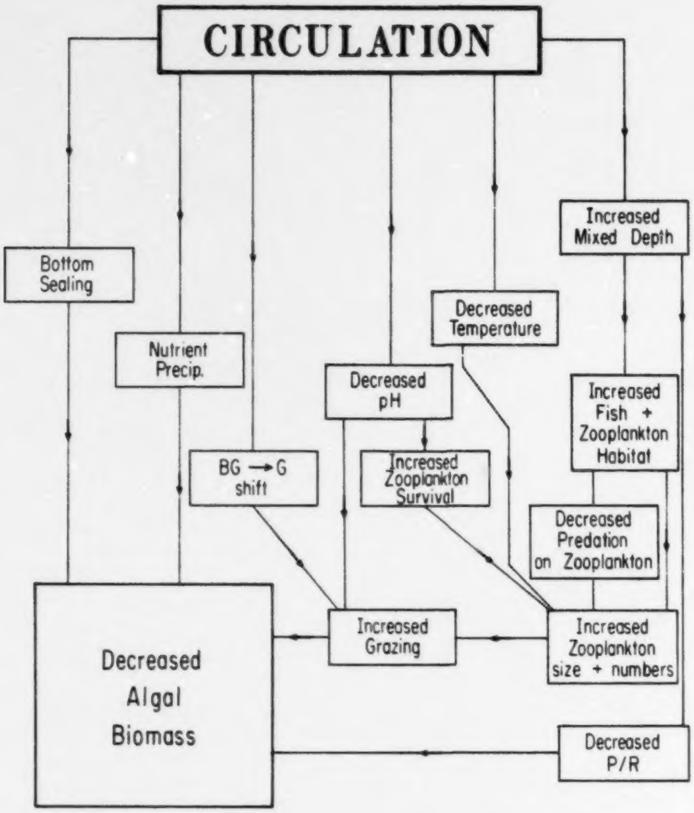

CIRCULATION
Increased Mixed Depth
Bottom Sealing
Decreased Temperature
Nutrient Precip.
Decreased pH
Increased Fish + Zooplankton Habitat
BG → G shift
Increased Zooplankton Survival
Decreased Predation on Zooplankton
Decreased Algal Biomass
Increased Grazing
Increased Zooplankton size + numbers
Decreased P/R

Fig. 14 a. A diagrammatic representation of the manner in which whole-lake circulation may act to reduce algal biomass.

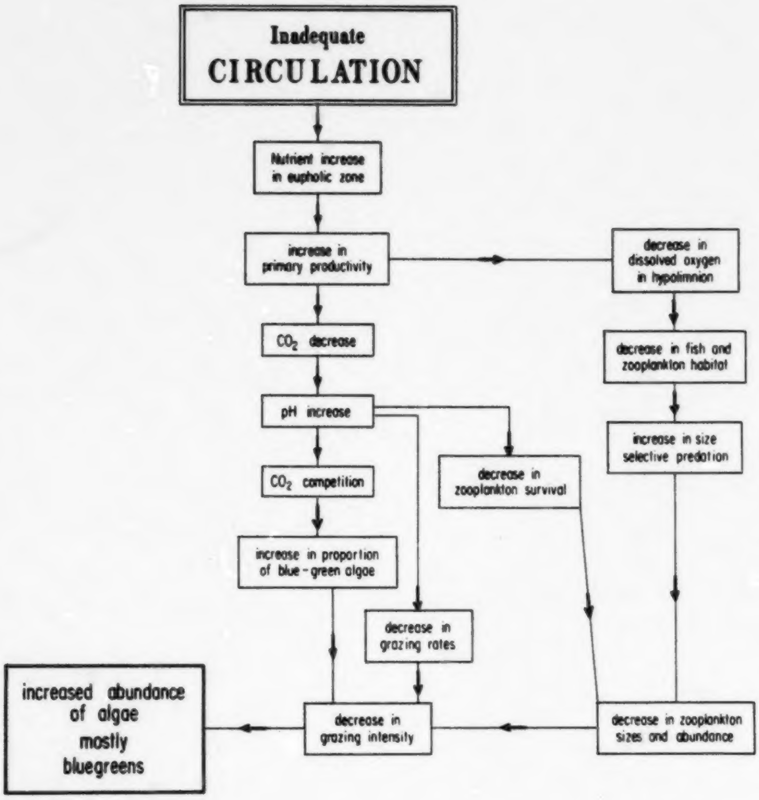

Inadequate CIRCULATION
Nutrient increase in euphotic zone
increase in primary productivity
decrease in dissolved oxygen in hypolimnion
CO_2 decrease
decrease in fish and zooplankton habitat
pH increase
increase in size selective predation
CO_2 competition
decrease in zooplankton survival
increase in proportion of blue-green algae
decrease in grazing rates
increased abundance of algae mostly bluegreens
decrease in grazing intensity
decrease in zooplankton sizes and abundance

Fig. 14 b. Possible ways by which inadequate circulation may result in increased abundance of algae.

P-K_2HPO_4
N-NH_4Cl
Alkalinity-$NaHCO_3$
CO_2-CO_2 gas

The final concentrations of these substances in the artificial hypolimnion could be controlled to simulate the natural hypolimnetic levels. Enclosures were mixed with diffused air released from a centrally located polyethylene air line. The rate of mixing and the depth of the mixed layer in each enclosure could be varied by adjusting the rate of air flow and the depth of air release, respectively (Fig. 15). Air flow was regulated at the surface with needle valves (Hoke, Inc.). The needle valves were connected to a manifold which received air from either an on-shore compressor (1/2 hp) or a compressed air tank on the raft. In a few enclosures carbon dioxide gas was also released at the diffuser head. After the first two experiments an attempt was made to remove planktivorous fish which were often trapped accidentally in the enclosures. Before the air was turned on each enclosure was slowly seined with a 1-meter diameter screen to remove any fish which might have been entrained. In one experiment planktivorous fish were also added to several enclosures to reduce the effects of herbivorous zooplankton. By varying the mixing rate, mixed depth, and additions it was possible to simulate a wide range of mixing conditions which could then be used in controlled experimental designs aimed at investigating specific response mechanisms. A more detailed description of each experimental design is presented in the text.

Sampling and Measurement

The enclosures were monitored at regular intervals (1-10 days). Special sampling techniques were employed to avoid disturbing the water column in stratified control enclosures. Temperature and oxygen profiles were obtained with a YSI probe. Transparency was determined with a Secchi disc which could be lowered side-ways and then flipped into a horizontal position to take the reading. Air flow was measured with a Gilson air flow meter either at the surface after disconnecting the air line from the needle valve (expts. 1, 2, 3, 4) or at 7 meters with an "in line" meter (expts. 5, 6, 7, 8). Water samples were taken at the surface in experiments 1 and 2 and with an integrated tube sampler (Lund 1949) in experiments 3-8. The depth of the water column sampled with the tube sampler was constant for each experiment but was varied between experiments so that the entire euphotic zone was usually sampled. Water from integrated samples was routinely analyzed for TP, alkalinity, pH, acid corrected chlorophyll a, and occasionally for chlorophyll b, chlorophyll c, NO_2, NO_3, NH_3, CO_2, and Particulate Organic Carbon. Particulate Organic Carbon, Chl a, Chl b, Chl c, were determined according to Strickland and Parsons (1968). TP was measured by ascorbic acid methods following persulfate oxidation (Menzel and Corwin 1965). The cadmium reduction technique (APHA 1971) was used to determine NO_2 and NO_3 and the Chaney and Marbach (1963) method was used to measure NH_3. Alkalinity was determined according to Standard Methods (APHA 1971). pH was measured with a Beckman pH meter immediately after the samples were taken. When CO_2 was added to artificial hypolimnia in closed enclosures the CO_2 concentration was determined by field titration (APHA 1971). CO_2 concentrations were also calculated from measurements of alkalinity, pH and temperature made on routine samples (Stumm and Morgan 1970). A subsample from each surface or integrated sample was preserved with Lugol's solution for algal counts; 10 mls of each subsample was concentrated by centrifugation and photographed at

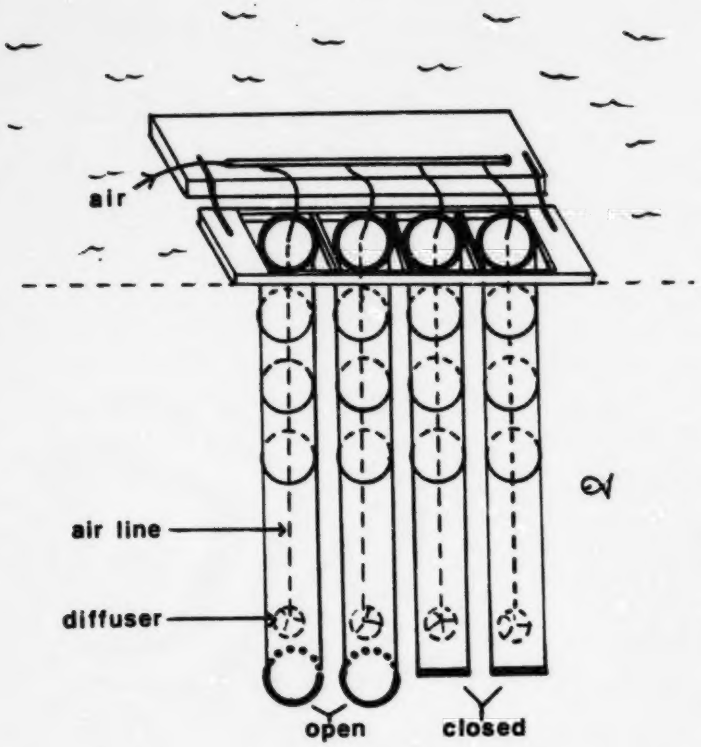

air
air line
diffuser
open
closed

Fig. 15. Experimental apparatus for suspending polyethylene enclosures.

100x and 400x with a Leitz Ortholux photomicroscopic system. These photomicrographs were used to identify sampling dates where major changes in species composition or abundance occurred. The phytoplankton on these dates were then counted using an inverted microscope (Utermohl 1958). This procedure greatly reduced the number of phytoplankton counts necessary to describe changes in the phytoplankton. Phytoplankton numbers were converted to algal volume using average cell volume values calculated for each species. The average cell volume was calculated by determining the dimensions of 10-100 cells, calculating the volume for each cell using a simple geometrical formula and then averaging the volumes. Cellular carbon for the autoradiographic analyses was calculated from cell volume using the regressions of Strathman (1967).

Rates of primary production were determined at both the community and species level in experiments 3 and 6. In experiment 3 samples were collected from several depths, spiked with C14-bicarbonate and incubated in duplicate 300 ml glass bottles, *in situ*. An initial subsample from each depth was also taken to determine concentrations of total inorganic carbon. This procedure was found to be too time consuming when incubations were carried out simultaneously in several enclosures. Consequently a different method was developed and applied in experiment 6. In this method an integrated water sample from the mixed layer was collected, spiked with C14-bicarbonate and incubated in duplicate 7 meter clear Tygon tubes (12 mm ID). (The depth of the euphotic zone was always less than 7 meters.) A subsample of the original integrated sample was also retained for determination of TP, Chl *a*, POC and total inorganic carbon. Aside from these differences, the procedures followed in both experiments were essentially the same. The incubations lasted from 1-4 hours at which point Lugol's solution was added to the incubation mixture to kill the phytoplankton. Community C14 activity was determined with the acid bubbling technique (Schindler *et al*. 1972) using an Amersham Searle scintillation counter. Total inorganic carbon and rates of carbon fixation were calculated by methods described in Bueltman *et al*. (1969). In experiment 3 the integral rate of photosynthesis (mg C m-2 d-1) for the community was determined by integrating the volumetric rates of photosynthesis (mg C m-3) found in the bottles with depth. In experiment 6 the integral rate of photosynthesis for the community was obtained by multiplying the volumetric rate of photosynthesis found in the Tygon tube (mg C m-3) by the length of the Tygon tube (meters). In a parallel incubation using both tubes and bottles the tube method gave an integral approximately 30% higher than the bottle method. This difference was most likely due to the inaccuracies inherent in the bottle technique. Since bottles were placed at discrete depths there was a high probability that the depth corresponding to the maximum photosynthetic rate was missed resulting in an underestimate of π. This was not a problem with the tube method since all depths are equally represented. Continuous light readings from a station 5 miles from the incubation site were used to calculate incident irradiance associated with an incubation period. Daily integral rates of photosynthesis were then determined from the product.

Daily integral photosynthesis =

$$\left[\begin{array}{c}\text{integral photosynthesis}\\ \text{during incubation}\end{array}\right] \times \left[\frac{\text{daily irradiance}}{\text{incubation irradiance}}\right]$$

Rates of photosynthesis were also determined at the species level using the technique of track autoradiography. Samples for autoradiographic analysis were taken from the Lugol's preserved incubation samples. In experiment 3, subsamples were taken from each depth and combined to give a single integrated sample. Incubation samples from experiment 6 were already integrated. Species specific rates of photosynthesis were then determined according to the method described by Knoechel and Kalff (1975). The photosynthetic activity determined from these integrated samples represents the average activity of the mixed layer population.

When possible, zooplankton tows were taken in the experimental enclosures at the end of each experiment. Zooplankton tows were also taken initially in the closed bottom bags before any additions were made. However, initial tows were not taken in the open bottom bags to avoid disturbing chemical stratification. Instead, a tow was taken in the lake shortly after the open bottom bags were lowered and this sample was assumed to be representative of the populations in the enclosures. Zooplankton samples were preserved with a sucrose-formalin solution (Haney and Hall 1973). The abundance of herbivorous species was determined by direct counts using a Sedgewick-Rafter cell at 100x.

Special Analytical Methods

Statistical procedures used throughout are taken from Snedecor and Cochran (1973).

The grazing potential associated with each zooplankton community was calculated using species filtering rates obtained from the literature. A regression of filtering rate on organism length was determined for cladocerans using the literature data ($r2 = .60$). This regression was used to calculate filtering rates for cladoceran species when literature values were not available. A list of species-specific filtering rates and the regression equation used in the calculation of community filtering rates is presented in Table 16.

Instantaneous algal growth rates were determined at both the species and divisions level in experiments 1 and 6 using the following equation:

$$r = \ln (A(t2)/A(t1))/(t2-t1) \qquad (12)$$

where r = instantaneous specific growth rate (day-1)
ti = sampling time i (days)
Ati = algal biomass at time i (biomass units m-2)

These instantaneous specific growth rates, r, should not be confused with the finite specific rates, R, B, and D, which were presented in the theoretical framework. Although both types of rates can be used to describe the growth of an algal population they are not interchangeable. The basic difference is that the finite rates are compounded at relatively large time intervals while the instantaneous rates are compounded at infinitesimally small time intervals. Algae grow more or less continually and algal growth is therefore best described by instantaneous rates. We have used instantaneous rates whenever possible in our analysis. It was necessary, however, to use finite rates at the community level in experiment 6 in order to evaluate the model presented in the theoretical framework. The finite specific growth rate, R, was calculated as:

$$R = \frac{A(t2) - A(t1)}{(t2-t1)\ \bar{A}} \qquad (13)$$

where A = the average level of algal biomass between sampling times 1 and 2 (biomass units m-2). The finite specific rate of production, B, was calculated as:

$$B = \frac{\bar{\pi}}{\bar{A}} \qquad (14)$$

where $\bar{\pi}$ = the average daily integral rate of photosynthesis between sampling dates (mg C m-2 d-1) and the finite specific loss rate was obtained by difference:

$$D = B - R \qquad (15)$$

Algal carbon was used as the biomass unit in these calculations. Algal carbon was determined as the product of the chlorophyll concentration and the carbon to chlorophyll ratio in the phytoplankton, θ.

EXPERIMENTS AND RESULTS

Limnology of Enclosures

Before the results of our experiments in polyethylene enclosures could be compared to those obtained in whole-lake circulation experiments, it was first necessary to demonstrate that the limnological environments inside and outside of the enclosures were sufficiently similar. The similarity of these environments was determined by comparing limnological conditions in the open bottom control enclosures (not mixed) with those found in the surrounding lake. Data from experiment 3 were used for this comparison. Figures 16a and 16b show the depth-time distributions of isotherms and isopleths of oxygen for Twin Lake during this experiment. The thermocline was fairly constant at approximately 5 meters. A metalimnetic peak in oxygen concentration is evident during the month of August. This maximum coincided with the development of a metalimnetic population of *Oscillatoria rubescens*. The disappearance of this oxygen maximum in late August coincided with a slight increase in the depth of the mixed layer. The portion of the metalimnion which was eroded at this time apparently contained the photosynthetically active segment of the *Oscillatoria* population. The depth-time distributions of isotherms and isopleths of oxygen for one of the open bottom control enclosures (bag 1) in experiment 3 are shown in Figures 17a and 17b, respectively. The distribution of isotherms in the control enclosure is virtually identical to that of the lake. The distribution of oxygen isopleths in the control enclosure is also quite similar to that of the lake indicating that the processes of atmospheric exchange and photosynthesis responsible for

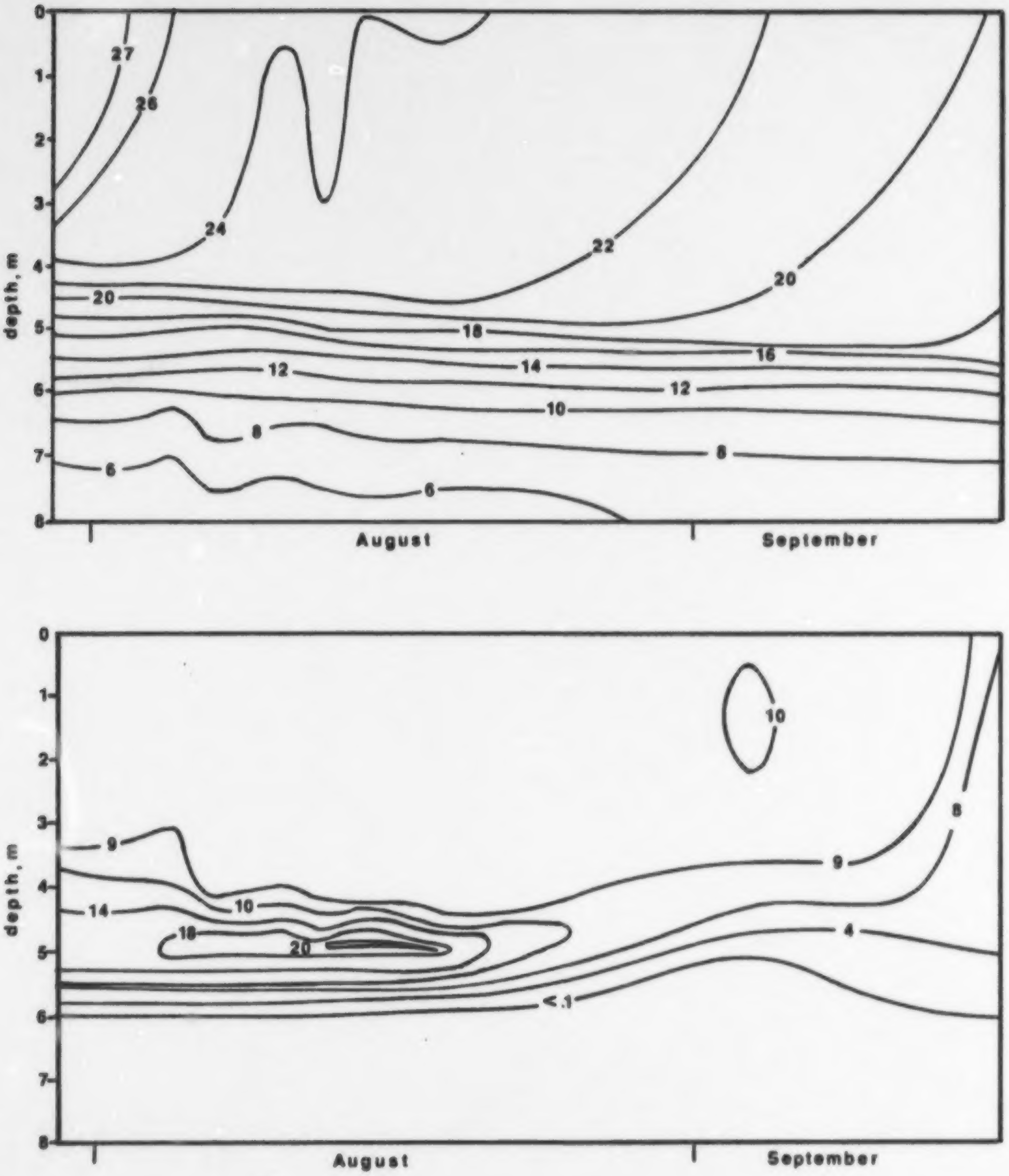


Fig. 16. Top,(a) isotherms (^{0}C) and bottom,(b) isopleths of oxygen in Twin Lake during experiment 3.

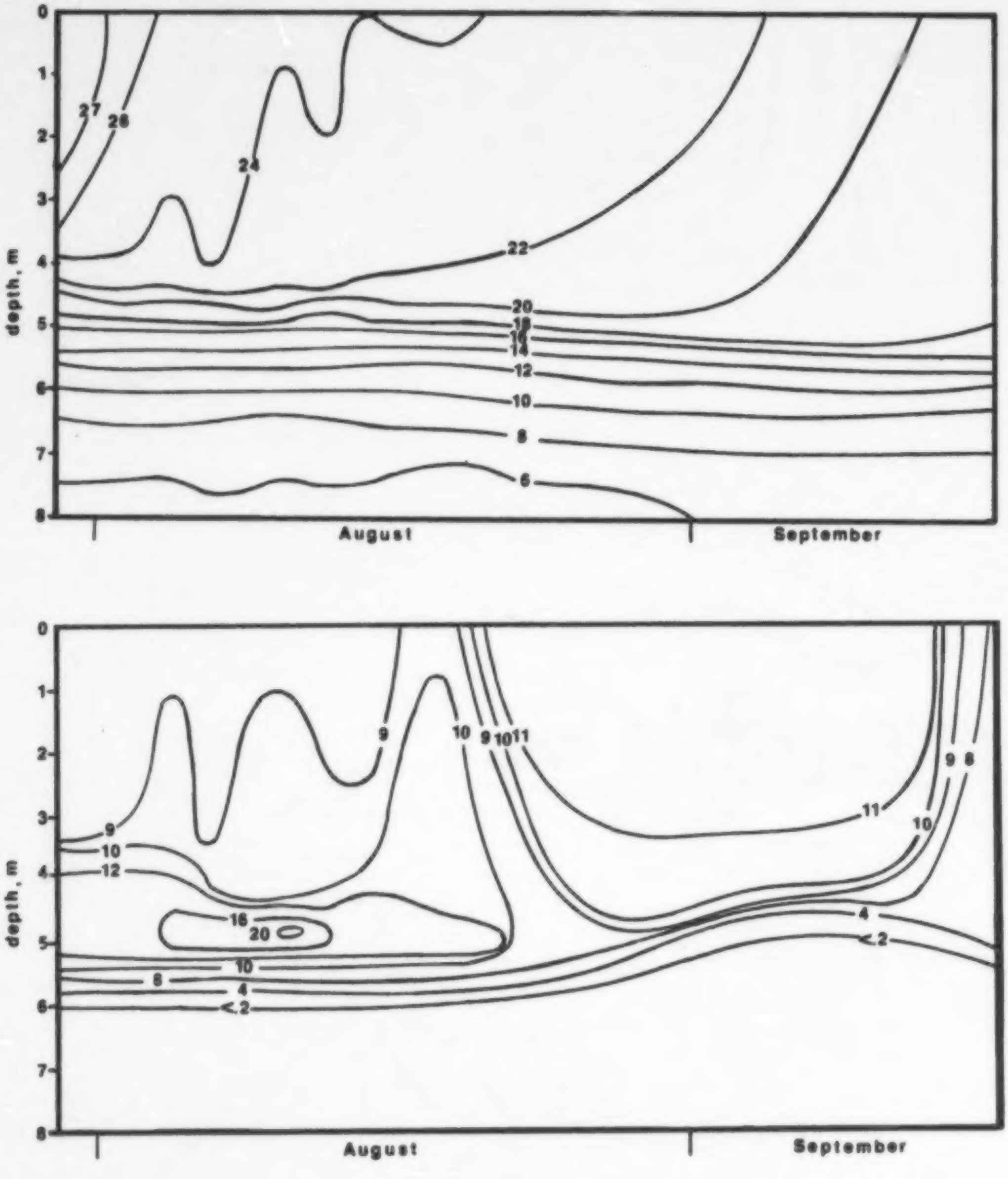


Fig. 17. Top, (a) isotherms (^{0}C) and bottom, (b) isopleths of oxygen in unmixed control bag 1 during experiment 3.

the observed oxygen distributions were similar in both environments. Significantly, the metalimnetic population of Oscillatoria and associated oxygen maximum which were found in the lake also developed in the control enclosure.

The disappearance of the oxygen maximum occurred at approximately the same time in both systems. A number of other important variables including Chl a, TP, Secchi depth, pH and CO2 also followed similar trends in both the control bag and the lake (Fig. 25). The correspondence between conditions in the lake and control enclosures was not as good in Little Lake Johanna. This is primarily a result of the high variability in limnological conditions characteristic of this lake. Little Lake Johanna receives a great deal of storm water runoff from an artificially enlarged drainage basin which during storms results in large fluctuations in lake chemistry and biology. Once the experimental enclosures were set in place the enclosed water columns were essentially isolated from these chemical and biological changes and limnological conditions within the enclosures tended to diverge from those in the lake. This was not a problem in Twin Lake because the drainage basin was small and the lake was relatively undisturbed.

It was also important to determine whether the changes in enclosure limnology resulting from artificial circulation were similar to those reported in destratified lakes and reservoirs. Data from experiment 3 were again used for this purpose. Figures 18a and 18b show the depth-time distribution of isotherms and oxygen isopleths in an open bottom enclosure circulated with compressed air which was delivered at a rate of 100 mls air/minute (measured at the surface). It is evident from the isotherm distribution that complete thermal destratification was not achieved at this mixing rate. This was surprising since this rate of air release per unit of lake surface area (.127 liters per minute/m-2) was almost 13 times the level recommended by Lorenzen and Fast (1976) (.01 liter per minute/m-2) to achieve destratification in lakes and reservoirs. Thermal stratification is apparently maintained in the mixed enclosure by efficient thermal conduction through the side walls. Flow rates in excess of 1000 mls air/minute were required to completely destratify the enclosures. However these higher mixing rates resulted in the resuspension of bottom sediments, which prohibited their use. Although thermal destratification was not achieved at a flow rate of 100 ml /min, Figure 18b indicates that sufficient mixing occurred to produce a uniform depth distribution of oxygen. This mixing rate was also found to produce a uniform distribution of algal cells, total phosphorus and pH in the mixed layer. Isometric depth distributions of chemical and biological variables are also characteristically found in destratified lakes and reservoirs (Toetz et al. 1972). We generally used the lower mixing rates (10-100 ml/minute) in our experiments in order to avoid resuspending sediments and also because the destratification of chemical and biological variables which was achieved at these rates was more important than thermal destratification in our theoretical framework.

During the experiments, there were certain effects on the phytoplankton which could be traced to the enclosures themselves. It will be important to distinguish these "enclosure effects" from the effects of the various treatments in each experiment. One direct effect of enclosure was to reduce turbulence in the water column. This tended to increase the effective sinking rates of the phytoplankton in the control enclosures which often had a lower algal standing crop at the end of an experiment than the lake. In addition to reducing turbulence, the walls of the enclosures also provided a substrate for the growth of periphyton. These periphyton competed with the phytoplankton for light and nutrients introducing some ambiguity into the

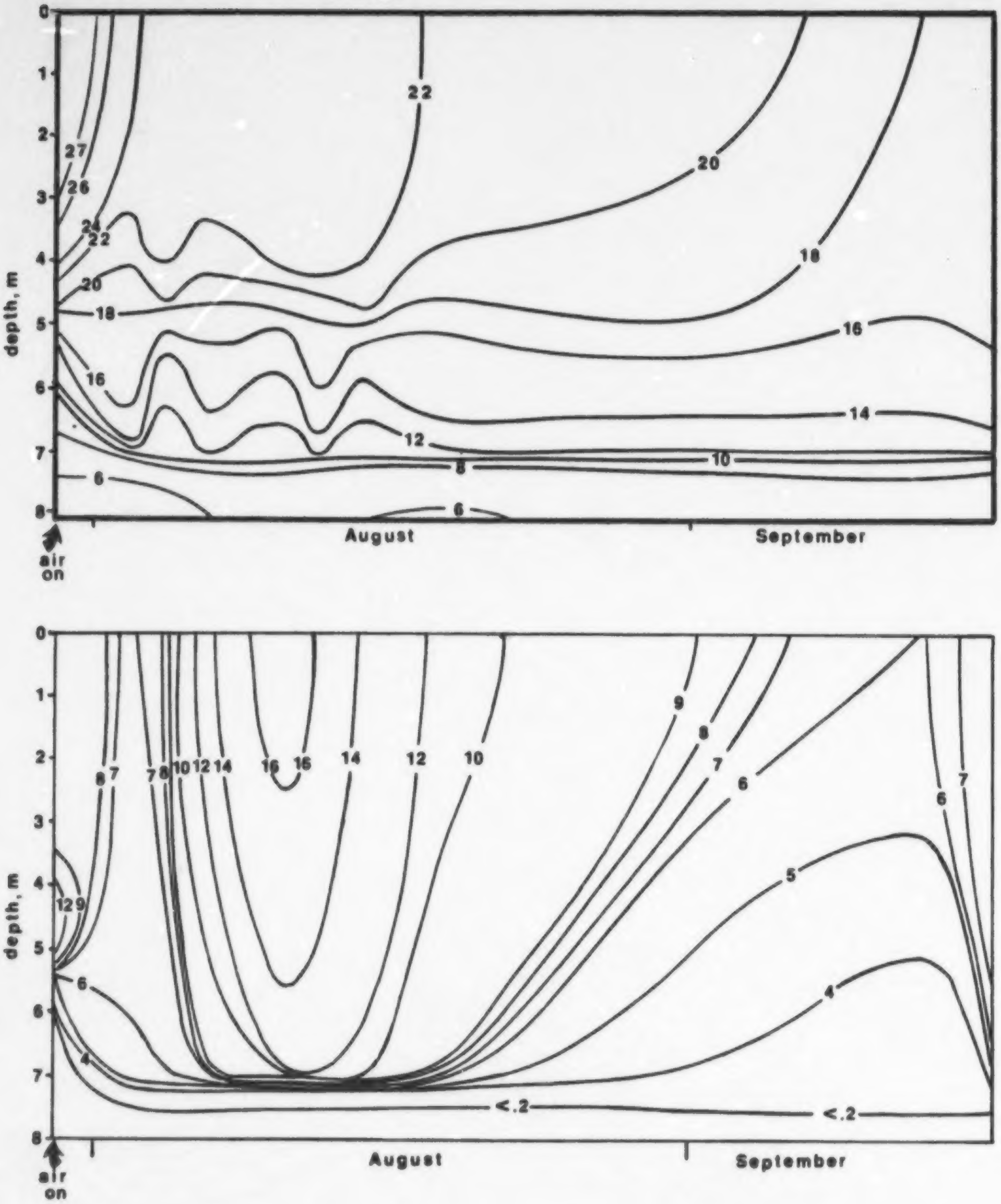


Fig. 18. Top, (a) isotherms (^{0}C) and bottom, (b) isopleths of oxygen in bag 1 (100 ml min^{-1}) during experiment 3.

interpretation of experimental results. The periphyton growth was most severe in the deep mixed enclosures because of the high nutrient levels produced in the euphotic zone. Periphyton growth was noticeable after the first two weeks in most experiments and changes in the phytoplankton which occurred after this period were therefore considered less significant. These and other "enclosure effects" will be addressed in the text when they influence the interpretation of experimental results.

Experiment 1

Our purpose in this experiment was to simulate the range of mixing conditions commonly found in artificially circulated lakes and to determine the response of the phytoplankton to these conditions. Open bottom enclosures were used to entrain "natural" water columns. Different mixing conditions were simulated by varying both the rate and the depth of mixing. The mixing rate was controlled by regulating the rate of air flow through the diffuser and the mixed depth was determined by the depth of air release. The shallow mixed depth was set at 3 meters to coincide with the depth of the mixed layer before circulation. This was done to prevent the introduction of hypolimnetic water into the mixed layer so that the direct effect of turbulence could be examined separately. The experiment was conducted on Little Lake Johanna during the period June 19 - June 28, 1976. Seven open bottom enclosures were suspended from two connected outriggers. The treatment associated with each enclosure is given in Table 7. Samples were taken at the surface and analyzed for TP, pH, alkalinity, CO2, and algal biomass, both as Chl a and algal volume. Temperature profiles, D.O. profiles and Secchi disc readings were also taken at regular intervals. Zooplankton tows were taken initially in the lake and in all of the enclosures at the end of the experiment. The depth of air release was raised to 7 m on June 22 in bags 2, 4, 5, and 7 when it was evident that sediments were being stirred up by the diffusers. The experimental results indicate that we were able to simulate a wide range of mixing conditions.

Complete thermal destratification was achieved in bag 7 at a mixing rate of 1000 ml/min. Unfortunately this rate also resulted in the resuspension of bottom sediments which continued even after the diffuser head was raised to 7 meters. The air release rate of 100 mls/min. in bags 4 and 5 was not sufficient to produce thermal destratification. However, this rate of mixing was rapid enough to produce a uniform depth-distribution of all chemical and biological parameters. The air release rate of 20 mls/min. maintained in enclosure 2 was too low to eliminate even chemical + biological stratification although considerable mixing did occur.

Changes in surface values of pH, CO2, TP, SD and algal biomass associated with each treatment are shown in Figure 19. There was an inverse relationship between CO2 and pH in this and all subsequent experiments which was due to interactions within the carbonate buffering system. There is also a consistent inverse relationship between S.D. transparency and chlorophyll concentrations in each experiment as predicted by the Lambert-Bouguer Law (Megard et al. 1979). CO2 increased and pH decreased as the mixing rate increased in deep mixed bags (2, 4, 5, 7). TP also increased in these enclosures as the mixing rate increased resulting in higher chlorophyll concentrations and lower transparency. The levels of CO2, pH, TP, and SD achieved at 1000 mg/min. (bag 7) were very similar to those attained at 100 mls/min. (bags 4 and 5) indicating that complete mixing occurred at 100 mls/min. Chlorophyll concentrations were lower in bag 7 than in bags 4 and 5 even though the TP levels were similar. This may indicate some inhibition of

Table 7: Treatments for enclosures in Experiment 1, Little Lake Johanna

Bag	Air on	Air off	Air Flow ($ml\ min^{-1}$ @ surface)	Air release depth (m)	Text designation
1	--	--	0	--	Control
2	6/19/76	6/28/76	20	8	Deep slow mixed
3	"	"	20	3	Shallow 20
4	"	"	100	8	Deep 100
5	"	"	100	8	Deep 100
6	"	"	1000	3	Shallow 1000
7	"	"	1000	8	Deep 100

algal growth at the highest mixing rate. The levels of most parameters in the shallow mixed enclosures (bags 3 & 6) did not differ greatly from the control and lake values, although chlorophyll a concentrations were somewhat lower in the control bag (bag 1). The average chlorophyll a concentration determined for each enclosure was an increasing linear function of the average TP concentration as indicated in Figure 20. (The numbers on this figure refer to the different enclosures.) The regression was significant at the .05 level. The fast-deep mixed enclosures all had relatively high average TP and chlorophyll values. The average Chl a value in the fastest mixed enclosure (7) fell below the regression line indicating possible inhibition. The control bag, shallow mixed bags, slow mixed bags and the lake all had relatively low average TP and Chl a values. The control bag (1) also fell below the line. Reduced turbulence in this enclosure may have acted to increase the effective sinking losses of the algae and lower the yield of chlorophyll. An increase in the mixed depth of the fast-deep mixed bags should have resulted in greater light limitation. The negative effect of light limitation on algal growth was apparently offset by the positive effect of increased nutrient availability in these bags.

Since the experiment was relatively short, the final species composition in each enclosure was generally similar to the initial composition (cf. Fig. 19) which made it difficult to determine the differential effects of treatment conditions at the division level. This problem was overcome by using the instantaneous specific growth rates for each division instead of the final species composition as an indicator of the algal response. Instantaneous specific growth rates were calculated for the diatoms, green and blue-green algae in each enclosure for the experimental time period of 10 days. The calculations were based on the change in algal volume summed for all species within a division. These growth rates are shown in Figure 21 along with average values of alkalinity, pH, CO2, temperature, TP measured and phosphorus availability measured at the surface during the same period. The ratio TP/Chl a was chosen as a measure of phosphorus availability since it was shown (Forsberg 1980) to be analogous to the cellular nutrient quota term, particulate phosphorus/chlorophyll a, used by Senft (1978).

There was a general increase in the growth rates for all divisions in the shallow mixed bags (3 and 6) as the mixing rate was increased. Although there were small increases in TP and CO2 in these bags, the average levels of phosphorus availability did not increase significantly over the control bag and this growth response apparently reflects the direct effect of increased turbulent mixing. While the mechanism involved is not clear, the effect of increased turbulence may have been to reduce the sinking rates of the algae. That the greatest stimulation in growth was found for the heavier diatoms seems to support this hypothesis. The additional effects of deep mixing can be seen in the results for bags 2, 4, 5, and 7.

Green algae and diatoms showed a similar response to deep mixing. Their growth rates both increased with the mixing rate reaching a peak at 100 ml/min and then declined again at the fastest mixing rate (bag 7). However, the diatoms showed a greater increase than the green algae. The blue-greens showed a different response pattern with their growth rates dropping steadily as the mixing rate was increased. The decline in growth rates for all divisions in bag 7 may have been related to the low surface temperatures produced in this enclosure by thermal destratification. (This may also explain the lower yield of Chl a found in bag 7 cf. Fig. 20.) With the exception of the results from bag 7, the growth rates of greens and diatoms generally exceeded those found at similar flow rates in the shallow mixed enclosures and this increase was probably due to the higher levels of

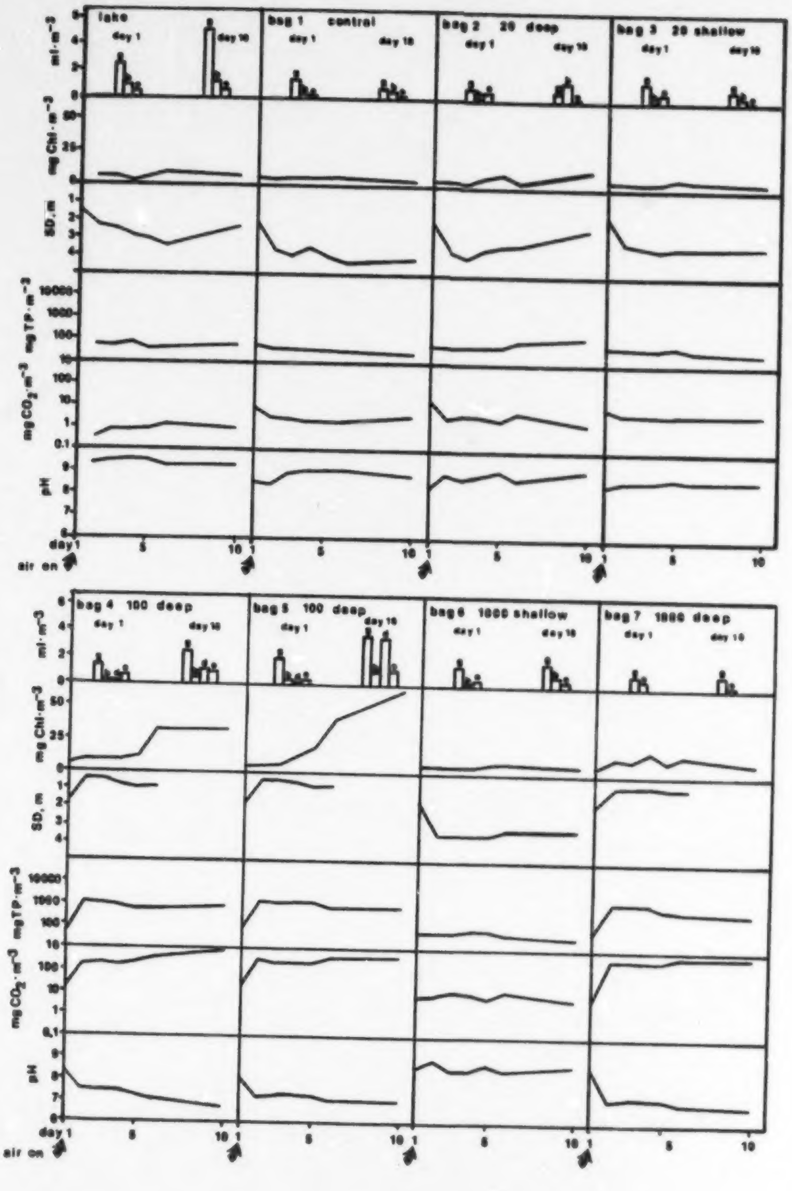


Fig. 19. Changes in pH, CO_2, TP, Secchi disc transparency chlorophyll a and algal volume in the experimental enclosures and in the lake during experiment 1, (g = greens, b = blue-greens, o = other).

nutrient availability observed in the deep mixed bags. A similar increase was found for blue-greens in the slow mixed enclosure (bag 2). However, the growth rate for blue-greens decreased at 100 mls/min (bags 4 and 5) in spite of high levels of nutrient availability. This inhibition of growth was apparently related to the low pH levels found at this mixing rate.

These results indicate that each division responds in a unique way to artificial circulation. This differential growth response can have an important effect on competitive relationships. Kalff and Knoechel (1978) have suggested that the competitive advantage of one group over another can be viewed as the difference between their observed growth rates. When the difference is positive the first group has the advantage because it grows at a faster rate but when the difference is negative the advantage shifts to the other taxonomic group. Applying this criterion to Figure 21 it is clear that diatoms maintained a competitive advantage over both greens and blue-greens in all of the enclosures. Similarly, blue-greens outcompeted greens under all treatment conditions. However, it should be noted that the competitive advantage of blue-greens over greens was dramatically reduced at the faster deep mixing rates. This latter shift in competitive advantage was the combined result of the stimulation of greens and inhibition of blue-greens found under these conditions.

The initial and final quantities of herbivorous zooplankton for experiment 1 are shown in Figure 22. There was a dramatic reduction in herbivore abundance in all enclosures by the end of the experiment. However, this response cannot be attributed to any treatment effect since the zooplankton also crashed in the lake.

In summary, the results from this experiment demonstrated the advantages of using a controlled experimental approach. We were able to create a wide range of mixing conditions resembling those commonly found in destratified lakes. The range of algal response associated with these conditions was also similar to that described in the destratification literature and the results were generally consistent with our theoretical framework. Slow deep mixing resulted in increased TP and pH levels at the surface. These conditions increased the competitive advantage of blue-green over green algae. Rapid-deep mixing resulted in low pH and high TP levels at the surface. These conditions increased the competitive advantage of diatoms over blue-greens and reduced the advantage of blue-greens over green algae. However, the blue-greens maintained a slight advantage over the greens so a quantitative shift toward greens was not observed. A general increase in total algal standing crop was correlated with an increase in TP in the rapidly mixed enclosures. This increase in biomass occurred despite an increase in the mixed depth indicating that the depressive effect of light limitation on algal growth was less important than the positive influence of nutrient enrichment. Although these results were encouraging several important limitations of the experimental design were encountered. Our inability to attain thermal destratification at relatively high mixing rates presented a serious problem. Because of the limitation we could not investigate the direct effect of thermal destratification on the algae. However it was possible to achieve isometric depth distributions of most chemical and biological variables and since these were the changes most likely to influence the phytoplankton we decided to continue using these flow rates in subsequent experiments. Another limitation of the experimental design was that it was difficult to separate the effects of different hypolimnetic factors such as CO2 and TP. This problem was primarily a result of using open bottom bags. The hypolimnion naturally contained a large number of chemical factors which could affect the

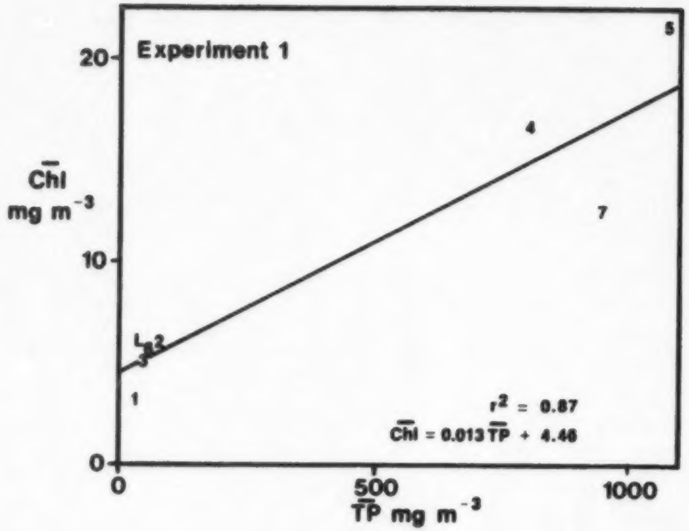
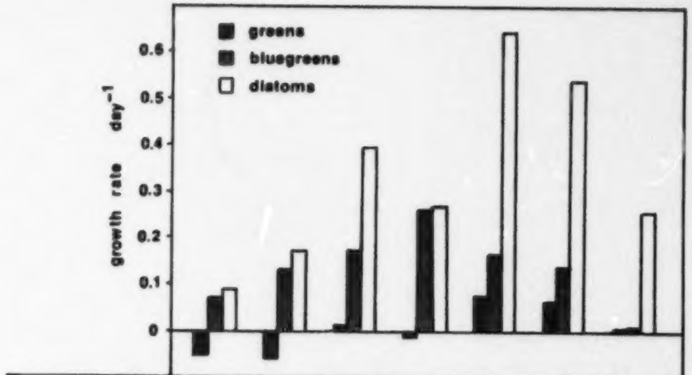


Fig. 20. Relationship between average concentrations of TP and chlorophyll a in the experimental enclosures and in the lake during experiment 1 (numbers refer to different enclosures, L = lake).



bag	1	3	6	2	4	5	7
flow rate ml·min^{-1}	0	20	1000	20	100	100	1000
z_m meters	—	3	3	7	7	7	7
pH	8.9	8.7	8.6	8.9	7.2	7.2	7.1
TP ug·l^{-1}	31	49	55	74	807	1096	946
Alk mg CaCO$_3$·l^{-1}	66	69	71	69	107	115	111
CO$_2$ uM·l^{-1}	2	5	8	3	227	294	370
Temp °C	24.5	23.2	23.0	24.2	22.7	23.0	12.4
TP·Chl^{-1}	9.0	9.3	8.8	11.5	43.5	31.3	66.7

Fig. 21. Composite instantaneous growth rates calculated in each enclosure for the first 10 days of experiment 1. Physical and chemical parameters shown represent the average values in each enclosure for the 10-day period.

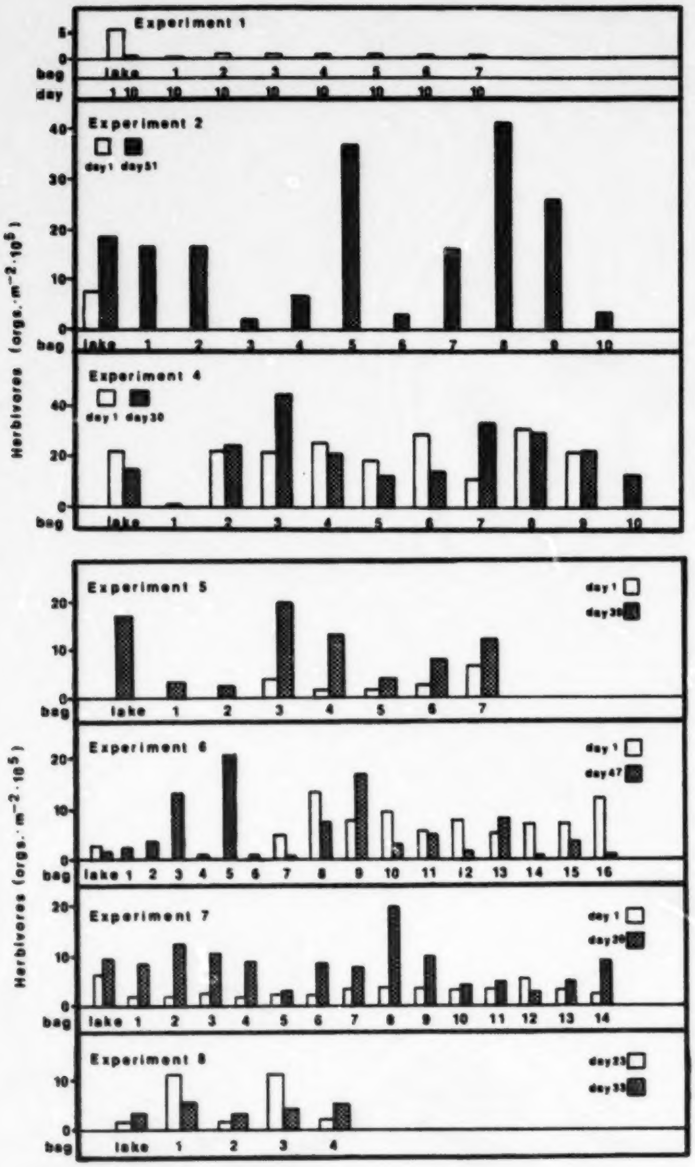


Fig. 22. Changes in the abundance of herbivorous zooplankton observed in the enclosures and lake during experiments 1, 2, 4, 5, 6, 7, and 8.

algae and these factors all tended to co-vary when they were brought to the surface during mixing. An attempt was made in later experiments to separate the effects of several of these factors using closed bottom enclosures and controlled hypolimnetic additions.

Experiment 2

The second experiment immediately followed the first (July 6 - Aug. 28, 1976) and was also carried out on Little Lake Johanna. The results of experiment 1 suggested that changes in pH, CO2 and TP during mixing were important factors in the algal response. Consequently, experiment 2 was specifically designed to investigate the response of the phytoplankton to changes in pH, CO2, and TP levels. A range of CO2, pH, and TP levels was created primarily by varying the air flow rate which determined the rate at which hypolimnetic TP and CO2 were introduced into the euphotic zone. CO2 was also added directly to some enclosures to obtain a very low pH. The influence of the rate of change in pH on the phytoplankton was also investigated in this experiment to test the hypothesis that a very rapid drop in pH might shock the blue-green algae resulting in some physiological damage perhaps making them more susceptible to cyanophage infection. To simulate this effect two of the enclosures (4 and 10) were circulated very rapidly for a brief period of time at the beginning of the experiment and were then allowed to restratify. All treatments are summarized in Table 8. Treatments were randomly assigned to the enclosures. Routine sampling procedures and analyses were identical to those used in experiment 1. Several treatment changes were made during the course of the experiment. Airflow was increased to 20 mls per minute in enclosures 2 and 7 on day 7 in an attempt to increase the mixing rate slightly. CO2 flow was increased to 150 mls. per minute in bag 6 on day 3 in order to produce a lower pH.

Trends in the surface values of chemical and biological variables in each enclosure are shown in Figure 23. As in the first experiment, pH decreased and CO2 increased at the faster deep mixing rates. The greatest drop in pH was observed in enclosure 16 which was supplied with additional CO2 gas. The pH in this bag dropped from 9.4 to 5.8 in the first seven days and remained low throughout the experiment. Once the pH dropped in the continuously mixed bags it remained low throughout the experiment. As expected, the most rapid pH drop was observed in bags 4 and 10. Thermal destratification was achieved after only 22 minutes in these enclosures. The observed drop in pH during this period was from 9.4 to 6.9 in bag 4 and from 9.4 to 7.0 in bag 10. CO2 and pH returned to control levels in these bags after only 5 days and remained there for the rest of the experiment. pH levels remained high in the slow mixed and control enclosures.

Surface values of TP also increased, as in the first experiment, with increases in the mixing rate. TP increased quickly in the rapidly mixed enclosures (4, 5, 6, 8, 9 and 10) and gradually in the slow mixed enclosures (2 and 7). Once the TP reached its peak in the continuously mixed enclosures it tended to decline very slowly. The decline was much faster in enclosures 4 and 10. This latter effect was most likely due to the quiescent conditions and presumably greater sinking rates which occurred in these bags after they restratified. As in the previous experiment, concentrations of chlorophyll a were correlated with TP. Figure 24 shows the linear relationship between average TP and Chlorophyll concentrations determined during the experimental period. The regression is just barely significant at the 5% level. Most of the variance in this relationship is due to the slow mixed bags (2 and 7). These enclosures were both dominated by the blue-green alga Microcystis

Table 8: Treatments for enclosures in Experiment 2, Little Lake Johanna

Bag	Air on	Air off	Air flow (ml min^{-1} @ surface)	CO_2 flow (ml min^{-1} @ surface)	Air release depth (m)	Text designation
1	--	--	0	--	--	Control
2	7/10/76	8/28/76	10(20)	--	7	20 deep
3	7/10/76	8/28/76	100	--	3	100 shallow
4	7/10/76 (22 min)	7/10/76	4000	1000	7	4000 deep instantaneous +CO_2
5	7/6/76	8/28/76	100	--	7	100 deep
6	7/10/76	8/28/76	50	50(150)	7	50 deep + CO_2
7	7/10/76	8/28/76	10(20)	--	7	20 deep
8	7/10/76	8/28/76	100	--	7	100 deep
9	7/10/76	8/28/76	50	--	7	50 deep
10	7/10/76 (22 min)	7/10/76	4000	--	7	4000 deep instantaneous

Table 9: Treatments for enclosures in Experiment 3, Twin Lake

Bag	Air on	Air off	Air flow (ml min^{-1} @ surface)	Air release depth (m)	Text designation
1	--	--	0	--	Control
2	7/30/76	9/20/76	100	7	100
3	"	"	20	7	20
4	"	"	100	7	100
5	--	--	0	--	Control
6	"	"	50	7	50
7	"	"	20	7	20
8	"	"	50	7	50

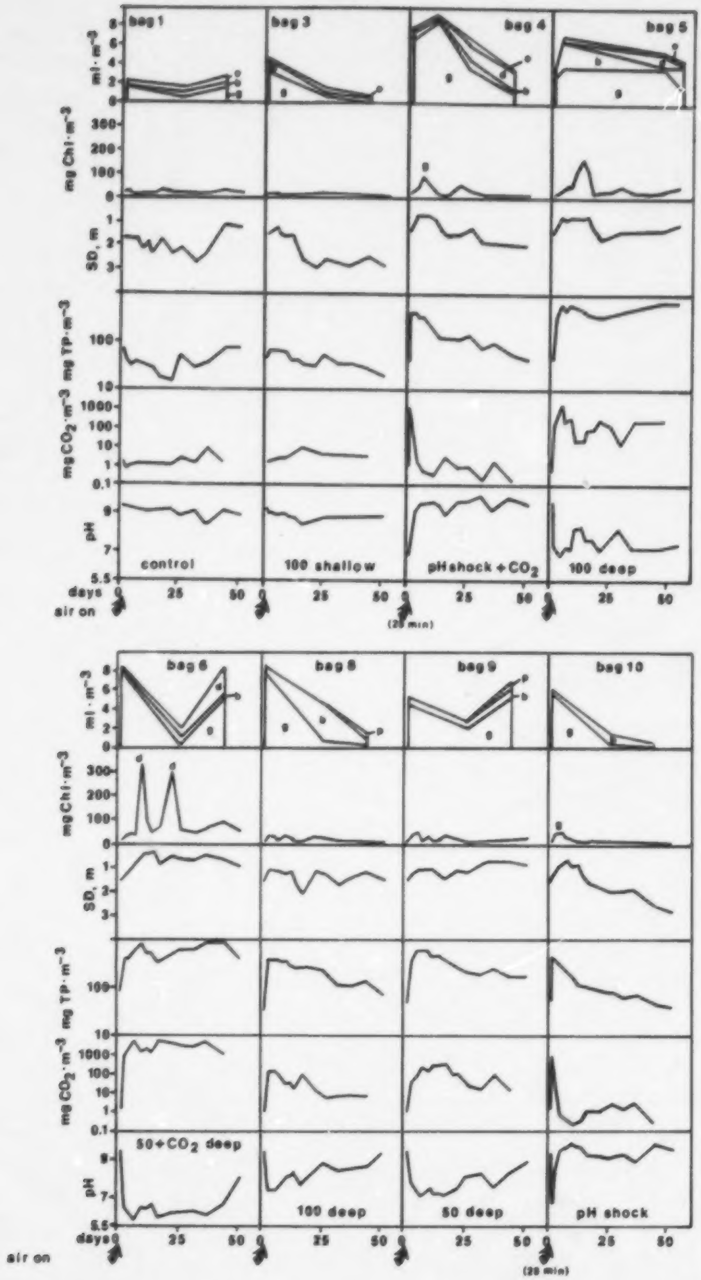


Fig. 23. Changes in pH, CO_2, TP, Secchi disc transparency, chlorophyll a and algal volume in the experimental enclosures and in the lake during experiment 2 (g = greens, b = blue-greens, d = diatoms, o = other).

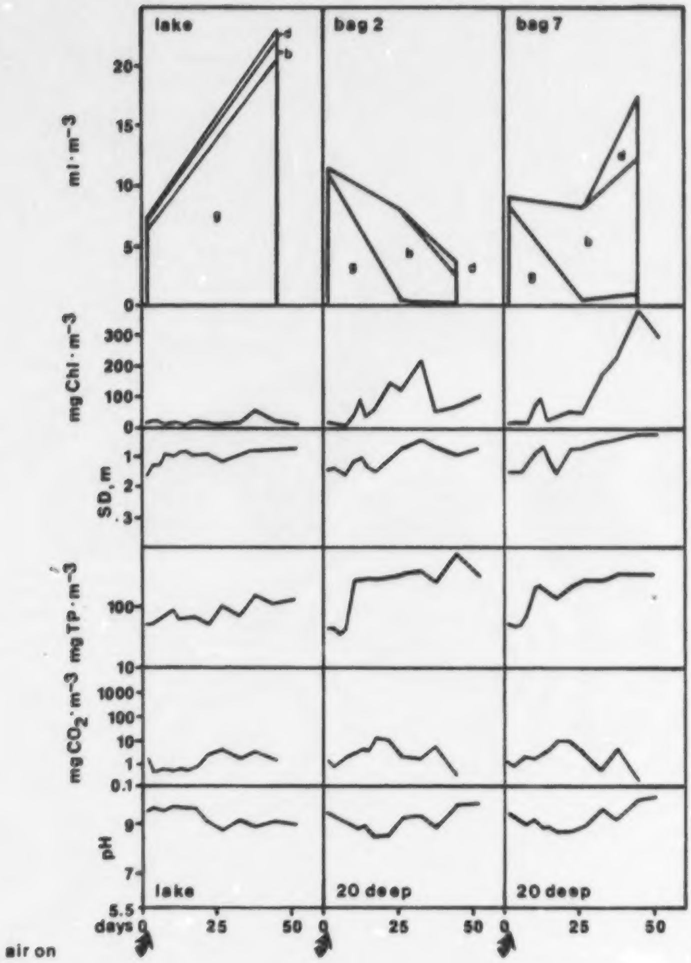


Fig. 23. Cont'd. Changes in pH, CO_2, TP, Secchi disc transparency, chlorophyll a and algal volume in the experimental enclosures and in the lake during experiment 2 (g = greens, b = blue-greens, d = diatoms, o = other).

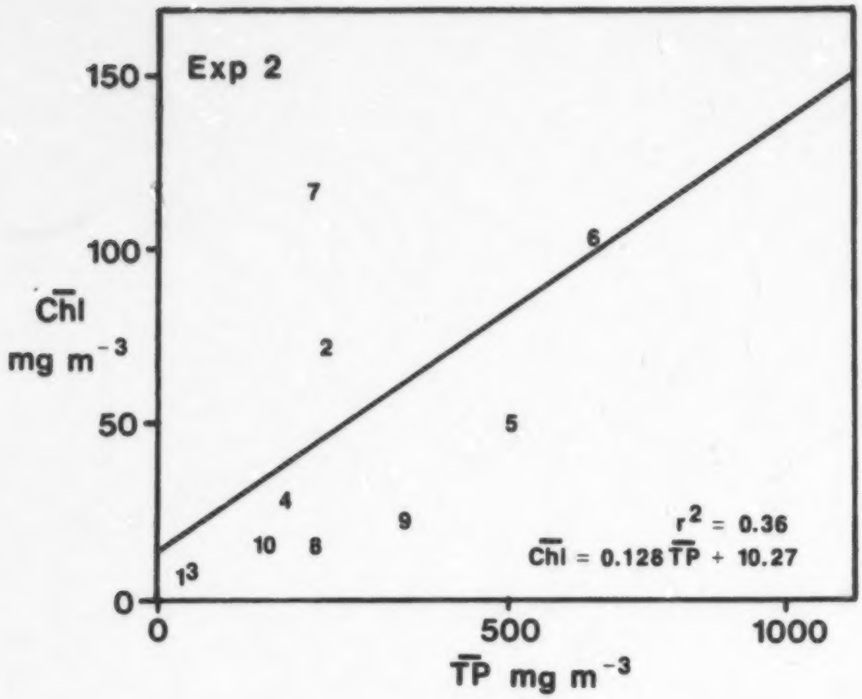


Fig. 24. Relationship between average concentrations of TP and chlorophyll a in the enclosures during experiment 2 (numbers refer to different enclosures).

aeruginosa which, due to its buoyancy, was able to accumulate near the surface. This resulted in a shallow effective mixed depth for this population which, according to equation 11 should have resulted in a higher yield of chlorophyll a per unit of total phosphorus. It is significant to note that bag 7, which deviated the most from the regression line also had the largest bloom of Microcystis aeruginosa. This experiment was long enough in duration so that changes in species composition associated with the different treatments could be easily discerned. However, caution had to be exercised in interpreting phytoplankton changes which occurred late in the experiment because bag effects such as periphyton growth (predominantly diatoms) became increasingly more important at this time. For this reason the analysis presented here will be restricted primarily to the first half of the experimental period. All enclosures were dominated initially by green algae (refer to Figure 23, predominantly by species ofSphaerocystis, Schroederia, and Coelastrum spp.). There was a shift to blue-green algae in the slow mixed bags (2 and 7), which had both high pH and TP levels. As indicated above, Microcystis aeruginosa became the dominant species in these enclosures. The response of blue-greens to low pH was inconsistent in this experiment. Blue-greens declined in bag 5 but only after an initial peak. The biomass of blue-greens remained fairly constant in bags 6 and 9 while their relative abundance increased dramatically in bag 8. There was some evidence that the rate of drop in pH may be important in determining the blue-green's response. Blue-greens were almost completely eliminated in bags 4 and 10 (determined from photomicrographs) shortly after the initial rapid drop in pH. However, they appeared to recover once the pH and CO2 levels had returned to the control levels. The response of the green algae to low pH was also less consistent than in the first experiment. There was a small peak of chlorophyll dominated by green algae shortly after the pH drop in enclosures 4 and 10 (determined from photomicrographs). These peaks disappeared, however, after the pH and CO2 returned to control levels. The relative abundance of green algae also increased in bag 5 but decreased in bags 6, 8, and 9 in spite of the low pH levels observed. Diatoms did not appear to out-compete the other divisions as well as they did in the first experiment. There were, however, two peaks of diatoms (identified from photomicrographs) in enclosure 16 which had the highest CO2 and TP levels. These peaks correspond to the two chlorophyll maxima shown in Figure 23. In addition, there was an increase in the relative abundance of diatoms in bag 5 which also had high CO2 and TP levels.

Changes in the abundance of herbivorous zooplankton in the experimental enclosures are shown in Figure 22. The zooplankton standing crop in the lake, the control (bag 1) and the slow mixed enclosures (2 and 7) all increased to approximately double the initial lake value. There was a decrease in herbivore standing crop in the shallow mixed bag (bag 3), the rapid mixed bag with the lowest pH (bag 6) and both bags which received the rapid pH drop treatment (bags 4 and 10). The greatest increase in herbivore standing crop occurred in the three rapid mixed bags which experienced only a moderate drop in pH (5, 6, and 9).

It is significant to note that the bags with the highest herbivore standing crop (5, 8, and 9) also fell considerably below the TP vs. Chl regression line in Figure 24 indicating that the lower yield (Chl/TP) in these bags may have been due to increased grazing pressure. Selective grazing on the more edible green algae may also have prevented the blue-green to green shift from occurring in bags 8 and 9.

Several of the results in this experiment were consistent with the theoretical framework. Slow deep mixing resulted in high surface values of TP and pH favoring the development of blue-green algae. Blue-green algae decreased and green algae increased shortly after a rapid drop in pH and increase in TP in bags 4 and 10. There was also a slow shift in relative abundance from blue-greens to greens associated with high TP and low pH in bag 5. At the community level, algal standing crop increased with TP in the mixed layer as predicted by equation 11. Low pH levels generally led to increases in zooplankton abundance. There were a number of results however, which did not appear consistent with our theoretical model. A shift in relative abundance from blue-green to green algae did not always occur in the bags with low pH and high TP levels. This may have been the result of the increased activity of herbivorous zooplankton in these bags. The zooplankton may have been selectively grazing the green algae which would have prevented the shift. This hypothesis was investigated in subsequent experiments. It was clear from experiment 1 that blue-greens can lose some of their competitive advantage to green algae at low pH and high nutrient availability and yet still remain competitively superior. This may explain the absence of a blue-green to green shift in some of the rapidly mixed enclosures in experiment 2. The response of zooplankton to low pH was also inconsistent. Extremely low pH apparently reduced zooplankton survival in bag 6. According to our framework (Fig. 14a) a reduction in pH should increase zooplankton survival. It is more likely, though, that there is probably an optimum pH for feeding and survival, below which survival declines. Evidence from the literature (Ivanova 1969; Kring and O'Brien 1976) suggests that this optimum is usually quite low. For example Ivanova found that the optimum pH for filtering in Ceriodaphnia was 7 and the rate drops steeply both above and below this point. It is quite likely then that the unnaturally low pH attained in bag 6 (low of 5.8) was below the optimum for most herbivores.

Experiment 3

Our purpose in experiment 3 was to determine whether the general response of the phytoplankton to artificial circulation differs significantly between lakes. Twin Lake, another small eutrophic lake was chosen for this experiment. The experimental design was similar to those in the first two experiments. Eight open bottom enclosures were suspended from two connected outriggers. The design was set up for four different treatments; unmixed (control), slow mix (20 mls/min), medium mix (50 mls/min) and rapid mix (100 ml/min). Each treatment was duplicated and all treatments were randomly assigned to enclosures. Treatment assignments are indicated in Table 9. Air was released from a depth of 7 meters in all enclosures. The experiment began July 30, 1976 and was terminated on September 20, 1976 after vandals destroyed the bags. Small planktivorous fish were occasionally trapped in enclosures as they were lowered during the first two experiments. These fish may have contributed to the variance in zooplankton and phytoplankton between bags. This problem was avoided in experiment 3 and all subsequent experiments by passing a screen through the enclosures several times after they were in place. This was done slowly to avoid disrupting stratification. Integrated water samples were taken with a tube sampler to a depth of 5 meters. This was the average thermocline depth in unmixed enclosures. Routine measurements and analyses were identical to those in the first experiment. In addition, species specific rates of photosynthesis were determined in several of the enclosures using the technique of autoradiography. (Refer to the Methods section for a discussion of this technique.). No zooplankton data were collected since it was not possible to obtain samples in the enclosures after they were vandalized.

Changes in pH, CO2, TP, SD, Chl a, and algal volume for each enclosure are shown in Figure 25. The pH dropped and CO2 increased in all of the mixed bags. The pH drop was approximately the same at all mixing rates falling from an initial value of 8.9 to approximately 7.9. The pH did not remain low in the mixed enclosures but returned to the high control levels shortly after the initial drop. The pH dropped again in all of the mixed enclosures towards the end of the experiment. TP and chlorophyll concentrations increased in all of the circulated enclosures. Again the average mixed layer concentrations of TP and chlorophyll were positively correlated. This relationship is shown in Figure 26. The regression is significant at the .05 level. The points corresponding to the fast mixed bags (2 and 4) seem to fall slightly below the regression line indicating a relatively low yield of chlorophyll per unit of TP and, perhaps, an inhibitory effect at these high mixing rates. There were very few changes in species composition associated with the different treatments. The phytoplankton community was dominated by Oscillatoria rubescens which usually accounted for more than 95% of the total algal biomass in all of the enclosures. In the stratified lake and control bags it occurred as a dense metalimnetic population while in the mixed bags it had a uniform depth distribution. The metalimnetic population in the lake and control bags were slowly eroded as the mixed layer increased naturally in the fall resulting in higher densities near the surface. The only significant change in species composition was found in bag 2 where a dramatic decline in Oscillatoria rubescens and a shift to green algae and diatoms occurred toward the end of the experiment. It should be noted, however, that periphyton growth on the side walls of this and other mixed enclosures became very dense toward the end of the experiment and most of the "planktonic" green algae and diatoms were periphytic forms. A decline in Oscillatoria was also observed in bags 4, 6, and 7 toward the end of the experiment but this species still remained dominant in these enclosures.

In situ C-14 incubations for the autoradiographic analysis were carried out in the lake and bags 1, 2, 4, 5, and 7 on August 18th. A series of 300-ml bottles with standard C-14 activity were incubated at 0, 2, 4, 5, and 6 meters for 2 hours. A single depth integrated sample was obtained from each set of bottles after the incubation and preserved for the laboratory analysis. Oscillatoria rubescens was the only species for which satisfactory track counts could be obtained. The other species were so rare that 95% confidence intervals were always greater than the mean track counts. Relative photosynthetic activity was expressed as DPM per 100 um of filament length. This should be interpreted as the average photosynthetic activity of Oscillatoria filaments in the water column. The results from this analysis are shown in Figure 27. The average activity of Oscillatoria rubescens in the lake and control enclosures was not significantly different. This result provides additional support for our assumption that the limnological environment within the enclosures is representative of natural lake conditions. There was also no significant difference in activity between the two rapidly mixed bags (2 and 4). The highest activity was found in the rapidly mixed enclosures while the lowest occurred in the control enclosures. The slow mixed enclosures (bag 7) had an intermediate level of activity. The higher activity in the mixed enclosures is difficult to explain. There was very little difference in pH between these enclosures at the time of the incubation. Greater nutrient availability might have led to higher specific photosynthetic activity but the ratio of TP/chlorophyll was actually lowest in the mixed enclosures. The most likely explanation for this difference in photosynthetic activity involves the stratification of Oscillatoria

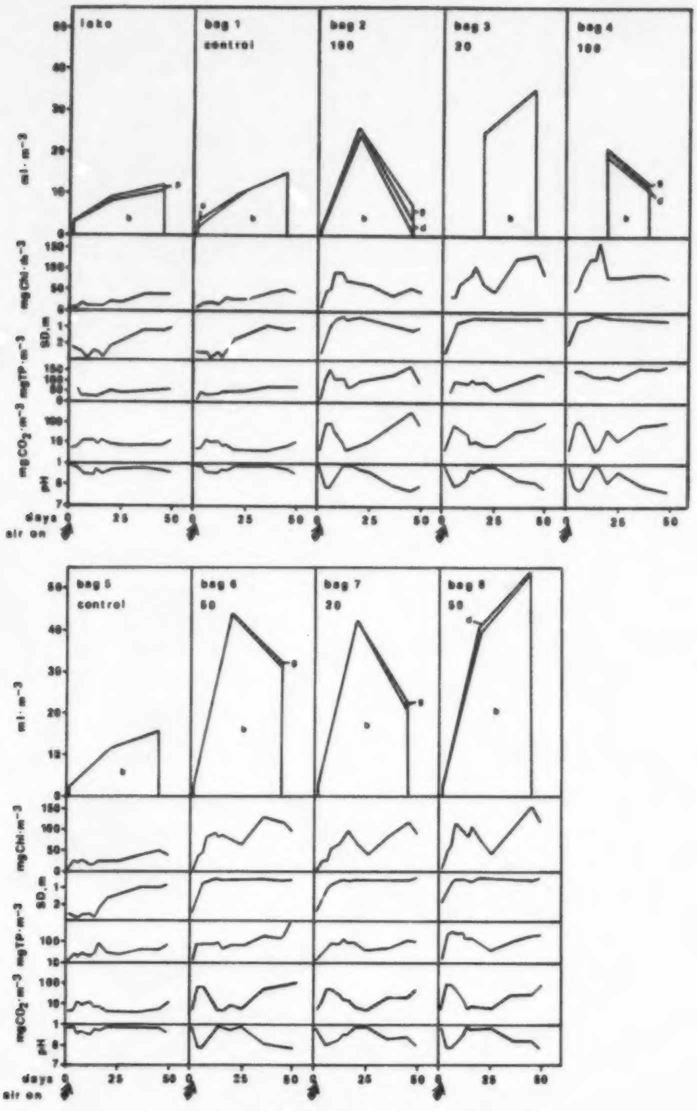


Fig. 25. Changes in pH, CO_2, TP, Secchi disc transparency, chlorophyll a and algal volume in the enclosures and the lake during experiment 3 (g = greens, b = blue-greens, d = diatoms, o = other).

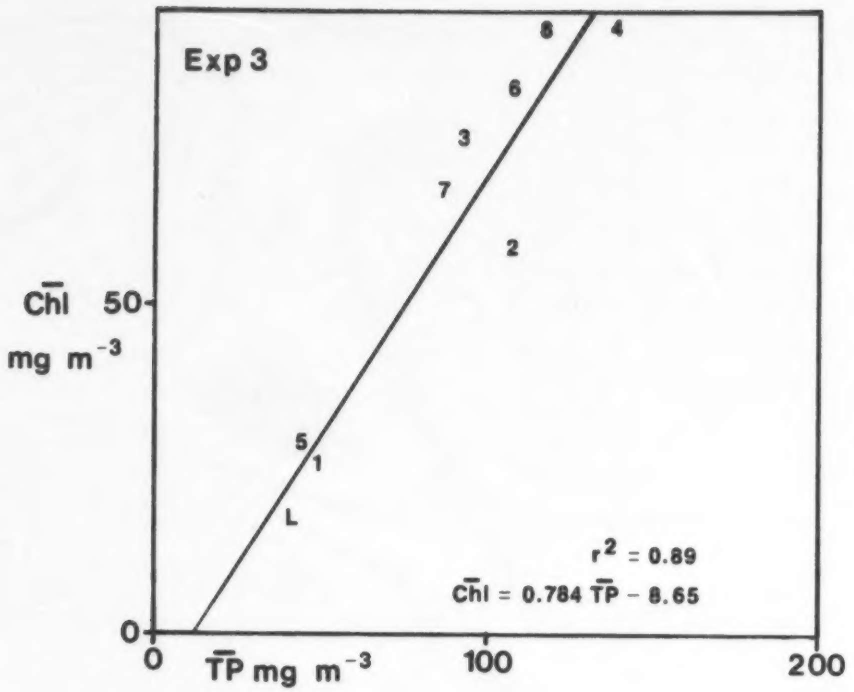
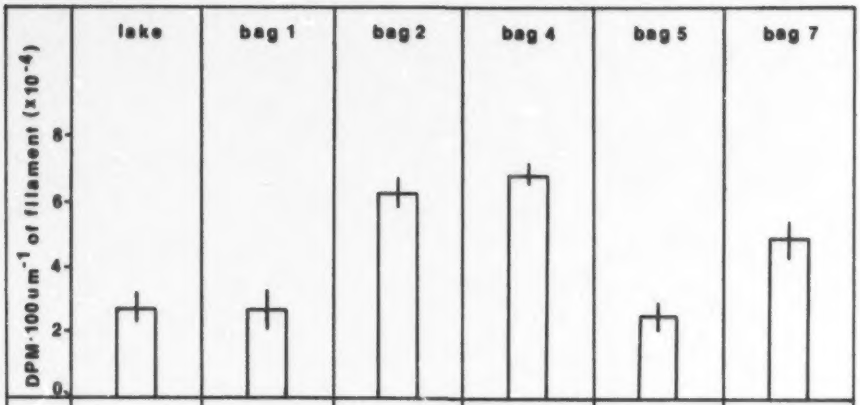


Fig. 26. Relationship between average concentrations of TP and chlorophyll a in the enclosures and the lake during experiment 3 (numbers refer to different enclosures, L = lake).



treatment	lake	control	100mls·min^{-1}	100mls·min^{-1}	control	20mls·min^{-1}
pH	8.63	8.92	8.72	8.08	8.93	8.87
TP/Chl	2.20	2.16	1.51	1.51	1.82	1.40

Fig. 27. Average photosynthetic activity of Oscillatoria rubescens determined autoradiographically in several enclosures and in the lake on August 18, 1976, experiment 3. Associated values of pH and phosphorus availability, TP/chl, are also indicated. (Vertical lines on bars represent 95% confidence intervals.)

populations in the control enclosures. Since the populations in the lake and control enclosures occurred at a depth near the bottom of the euphotic zone their rate of photosynthesis was probably light limited. The principal effect of mixing, then, was to bring these populations in contact with the higher light intensities near the surface. Therefore, the increased activity in the mixed enclosures was probably a result of greater light availability. This may also explain the lower nutrient availability TP/Chl in the mixed enclosures. Without the constraints of light limitation the algae were free to grow until nutrients became limiting. This may, in part, explain why higher algal standing crops were observed in the mixed enclosures.

Several of the results of this experiment were inconsistent with both our theoretical framework and, also, with the results of the previous two experiments on Little Lake Johanna. Mixing did result in a decrease in pH and an increase in TP levels as expected but a clear shift from blue-green to green species only occurred in one enclosure and that result is suspect because of the potential influence of periphyton. This shift did occur in the first two experiments and the important difference may be that the actual drop in pH associated with mixing was much greater in Little Lake Johanna than it was in Twin Lake. This difference was partly a result of the higher alkalinity and, hence, greater buffering capacity of Twin Lake water. The alkalinity in mixed Little Lake Johanna enclosures was in the range of 1.5 - 2.5 meq/l, which compares with 2.1 - 3.3 meq/l for Twin Lake. A second reason for the larger pH drop in Little Lake Johanna enclosures was that the level of CO2 was much higher in these bags. The CO2 concentrations in Twin Lake mixed bags seldom reached 100 ug/l while levels in mixed Little Lake Johanna bags often exceeded 500 ug/l. Both the initial alkalinity and CO2 levels in the hypolimnion, then, determine the pH drop following destratification. The high alkalinity and low CO2 levels in Twin Lake may also explain the rapid return to high pH levels after the initial drop in the mixed enclosures. According to our theoretical framework, light limitation should increase as the mixed depth increases. The exact opposite effect was observed for Oscillatoria in the mixed enclosures in experiment 3. This result occurred because a fundamental assumption of our theoretical growth model (equation 10) was violated. The model assumes a uniform distribution of algae in the mixed layer but the Oscillatoria population in Twin Lake was concentrated in the metalimnion. The effect of mixing then was to reduce light limitation instead of to increase it.

Some results at the community level in experiment 3 agree with those found in the previous two experiments. An increase in TP in mixed enclosures and a positive correlation between TP and Chl a were observed in all three experiments. However, the slope of the TP vs Chl a regression for experiment 3 was much steeper than those observed for the first two experiments. This may have resulted from the combined effect of nutrient enrichment and a release from light limitation on algal growth in the mixed bags in experiment 3.

Experiment 4

The results of the first three experiments suggested that changes in CO2, TP, and other nutrients during mixing might be influencing the phytoplankton response. However these results were somewhat ambiguous since it was not possible to separate the effects of these factors from other hypolimnetic factors which covaried in the open bottom enclosures.

Experiment 4 was the first of three experiments which utilized closed bottom enclosures and controlled chemical additions in an attempt to separate the effects of phosphorus, nitrogen and CO_2 from other hypolimnetic factors during circulation. The experiment was carried out from September 1 to September 30, 1976 on Little Lake Johanna. Eight closed bottom enclosures were used. Various combinations of phosphorus (as K_2HPO_4), nitrogen NH_4Cl) and carbon dioxide (gas) were added to the artificial hypolimnia of these bags in amounts designed to simulate natural hypolimnetic concentrations. CO_2 was added by bubbling samples of hypolimnetic water until they were presumed saturated and then returning them to the hypolimnion. The additions were varied between enclosures to produce a range of pH and nutrient levels so that the effects of these two factors could be separated. $NaHCO_3$ was also added to most of the enclosures to simulate the expected alkalinity in the mixed lake. All additions were made before the air was turned on. Open bottom bags were also included in the experimental design so the closed bottom bags could be compared to a less controlled natural system. One of these bags was not mixed and served as the control for both open and closed bottom bags. Air was released at 7 meters at a rate of 100 mls/min (measured at surface) in all mixed bags. A summary of treatments is given in Table 10. Integrated water samples were taken to a depth of 3 meters which was the approximate depth of the thermocline in the lake. Routine measurements and analyses were the same as those described for experiment 1 except for the determination of algal biomass. Algae were not counted and, therefore, algal volume was not determined in this experiment. Chlorophyll *a* and chlorophyll *b* provided the only quantitative measures of algal biomass. Chlorophyll *a* was assumed to represent total algal biomass. Chlorophyll *b* only occurs in the Chlorophyta and euglenoids. Since euglenoids were never observed in any of the enclosures the concentration of chlorophyll *b* was assumed to be a measure of the biomass of green algae. As in previous experiments, changes in species composition were also determined from photomicrographs of the algal samples. Zooplankton tows were taken initially in the lake and in all of the closed bottom bags. Final tows were taken in all bags and in the lake. All bags were also screened initially to remove fish. Changes in pH, CO_2, TP, SD and algal biomass in the enclosures are indicated in Figure 28. A rapid drop in pH was observed in all of the closed bags which had CO_2 additions (bags 3, 6, 8, 9) with the greatest drop occurring in the bag with the largest CO_2 addition (bag 9). The pH in bag 9 fell from an initial value of 8.6 to 7.0 one day after the air was turned on. The pH in the mixed open bottom bag (bag 10) also fell rapidly reaching a low of 7.2. Unfortunately, the pH was also low in the other bags which did not receive CO_2 as well as in the lake for most of the experiment. TP increased in all of the closed bottom bags which received nutrient additions (3, 4, 7, 8, 9) but the maximum level reached (approximately 380 ug/l) was considerably less than the peak observed in the mixed open bottom bag (bag 10, 647 ug/l). The level of TP remained high in the closed bottom-nutrient addition bags but dropped rapidly in the open bottom-mixed bag. TP levels in the bags which did not receive nutrient additions were similar to the control and lake levels. Chlorophyll *a* peaked in all enclosures and in the lake on day 6. This peak was followed by a rapid decline which was also independent of treatment conditions. The synchrony of the peak and crash in all of the bags and in the lake indicates that some common factor was responsible for this change. Since the experiment was begun late in the growing season the decline in chlorophyll *a* may have been the result of cooler temperatures or shorter day length. This may also explain why TP and Chl *a* concentrations were not as well correlated in this experiment as they were in the previous three. The relationship between average chlorophyll *a* and TP concentrations in all enclosures is shown in Figure 29. The regression is not significant at the .05 level.

Table 10: Treatments for enclosures in Experiment 4, Little Lake Johanna

Bag	Air on	Air off	Air flow (ml min^{-1} @ surface)	Air release depth (m)	Bag Type	Additions*	Text designations
1	--	--	0	--	open	--	open control
2	9/4/76	9/30/76	100	7	closed	A	closed high pH
3	"	"	100	7	closed	A+N+P+lowC	closed nutrients low pH
4	"	"	100	7	closed	A+N+P	closed nutrients high pH
5	"	"	100	7	closed	none	closed mixed control
6	"	"	100	7	closed	A+N+P+lowC	closed nutrients low pH
7	"	"	100	7	closed	A+N+P	closed nutrients high pH
8	"	"	100	7	closed	A+N+P+lowC	closed nutrients low pH
9	"	"	100	7	closed	A+N+P+highC	closed nutrients very low pH
10	"	"	100	7	closed	--	open mixed

*A(Alkalinity,NaHCO$_3$), N(Nitrogen, NH$_4$Cl), P(Phosphorus, K$_2$HPO$_4$), C(CO$_2$)

Table 11: Treatments for enclosures in Experiment 5, Little Lake Johanna

Bag	Air on	Air off	Air flow (ml min^{-1} @ 7m)	Air release depth (m)	Bag Type	Additions*	Text designation
1	7/8/77	8/15/77	50	7	open	--	open mixed
2	--	--	0	--	open	--	open control
3	"	"	50	7	closed	A+C	closed low pH
4	"	"	50	7	closed	A+N+P	closed nutrients high pH
5	--	--	0	--	closed	none	closed control
6	"	"	50	7	closed	A+N+P+C	closed nutrients low pH
7	"	"	50	7	closed	A	closed high pH

*A(Alkalinity, NaHCO$_3$), N(Nitrogen, NH$_4$Cl), P(Phosphorus, K$_2$HPO$_4$), C(CO$_2$)

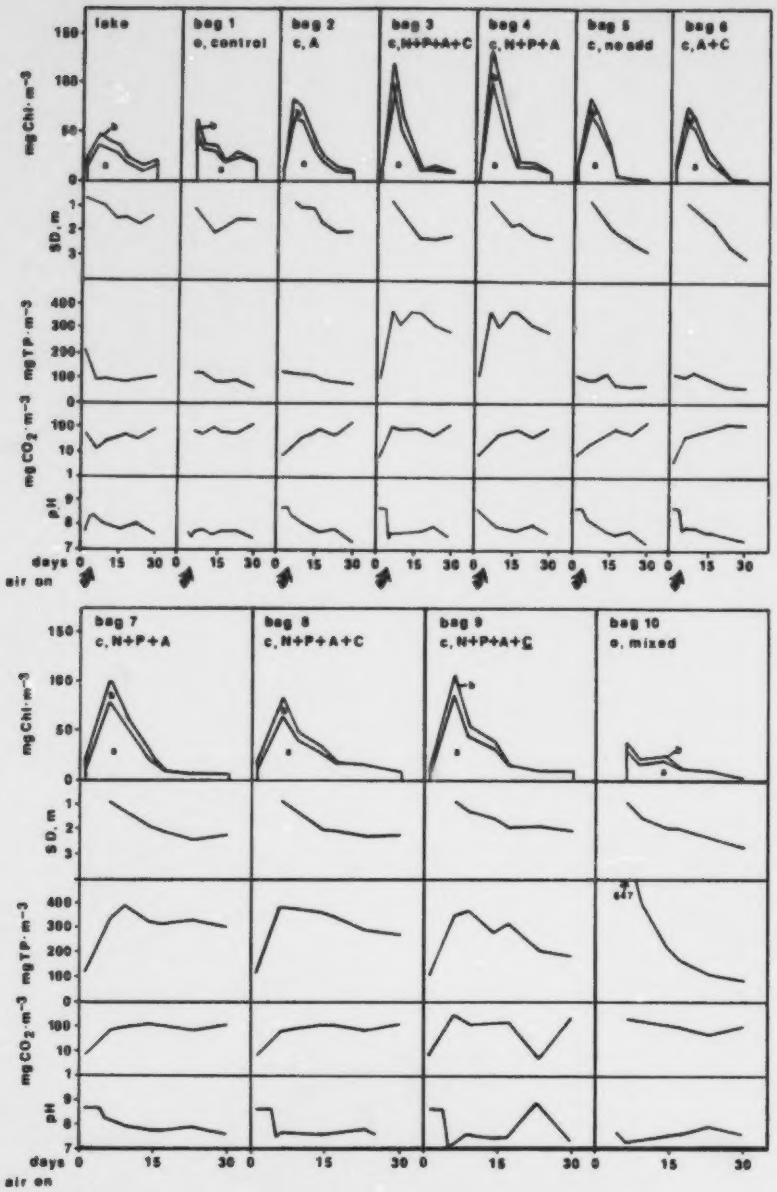


Fig. 28. Changes in pH, CO_2, Secchi disc transparency, chlorophyll *a* and chlorophyll *b* in the enclosures and lake during experiment 4 (a = chlorophyll *a*, b = chlorophyll *b*; treatment designations: o = open bottom, c = closed bottom, N = nitrogen, P = phosphorus, A = alkalinity, C = low CO_2, $\underline{C}$ = high CO_2).

There was only a slight increase in the average chlorophyll concentrations in the closed bottom bags which received nutrient additions. The yield in the mixed open bottom enclosure (10) was particularly low. This suggests that some factor other than mixing, TP, pH, alkalinity or CO2 may have inhibited algal growth in this bag. There were no major changes in species composition during this experiment. The flora in all enclosures and in the lake was dominated by green algae throughout the experiment and the green algae were not replaced by other species after their populations crashed. There was also no consistent effect of circulation on the abundance of herbivorous zooplankton. The changes which occurred in each enclosure are shown in Figure 22. When these zooplankton numbers were converted to community grazing rates (see Methods section), however, it was evident that the herbivores were influencing the final yield (Chl a) in the different enclosures. Figure 30 shows the relationship between the ratio TP/Chl a (inverse of yield) and the community grazing rate calculated on the day of the final zooplankton tow for all of the enclosures. The numbers on the graph again represent the different enclosures. The regression is significant at the .05 level. It is apparent from this Figures that those enclosures with the highest grazing rates also had the lowest yield.

This experiment primarily served as a test of the new controlled experimental design. We were able to separate the effects of pH, CO2, TP, nitrogen, alkalinity, physical mixing, and light limitation from the effects of a large number of unknown factors associated with the hypolimnetic milieu. However, we were not entirely successful in simulating natural levels of controlled substances. We were able to reproduce the drop in pH and increase in CO2 observed in the open bottom mixed bag but our nutrient additions were apparently insufficient to simulate the natural increase in TP observed during mixing. The response of the biota in this experiment was somewhat anomalous. However, we did not expect the results to be very meaningful since the experiment was begun so late in the growing season. Perhaps, the most significant result was the extremely low yield observed in the open bottom mixed enclosure. This result provides evidence for the existence of an unknown hypolimnetic factor, not adequately addressed in our theoretical framework, which has an inhibitory effect on algal growth.

Experiment 5

Experiment 5 was carried out on Little Lake Johanna between July 8 and August 15, 1977. As in the previous experiment closed bottom enclosures and controlled chemical additions were used to separate the effects of N, P and CO2 from other hypolimnetic factors during mixing. Open bottom enclosures were also used for comparison. Hypolimnetic additions were then varied between these enclosures in a factorial design to separate the effects of nutrients (N and P) and pH (CO2) during mixing. Two levels of nutrients (high N+P, low N+P) and two levels of pH (high pH, low pH) were used as indicated below.

	low pH	high pH
low N + P	Bag 3	Bag 7
high N + P	Bag 6	Bag 4

The calculations and procedures involved in the addition of hypolimnetic

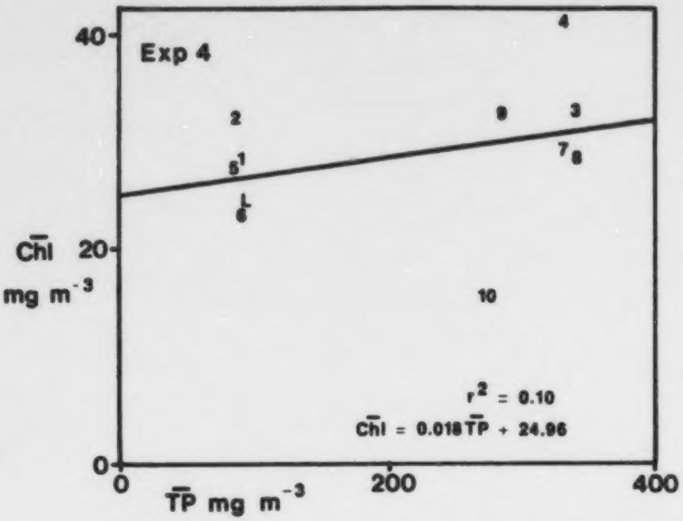


Fig. 29. Relationship between average concentrations of TP and chlorophyll a in the enclosures and lake during experiment 4 (numbers refer to different enclosures, L = lake).

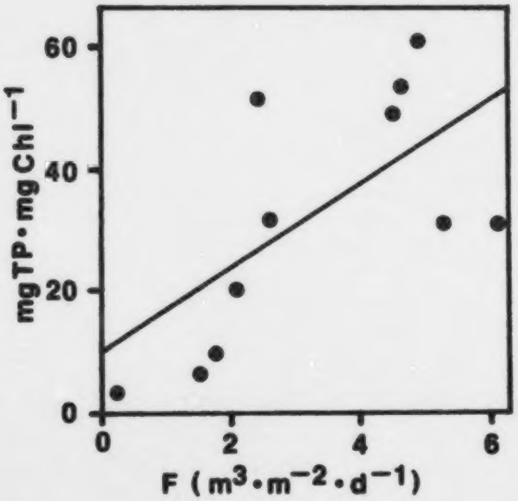


Fig. 30. Relationship between the total herbivore filtration rate (F) and the ratio TP/chlorophyll a in the enclosures and lake on the final day of experiment 4.

nutrients to the closed bottom bags were modified slightly in experiment 5. Integrated samples were taken to a depth of seven meters (the final mixed depth) in both open and closed bottom enclosures and analyzed to determine what the concentrations of N, P, CO2 and alkalinity would be after mixing. Additions of N, P, CO2 and alkalinity to the closed bottom bags were then calculated so that the final mixed-concentration would be the same as the average mixed-concentration found in the open bottom enclosures. Nutrients were added to the hypolimnia in the same manner as in experiment 4 except for CO2 which was added in the following way. Hypolimnetic water was first collected from a closed bottom bag and placed in a container of known volume. The concentration of CO2 was determined initially and then again after the water was bubbled with CO2 gas to determine the exact quantity of CO2 added before the water was returned to the hypolimnion. In this way we could accurately control the amount of CO2 added to each hypolimnion.

Air was released at a depth of seven meters in all of the mixed enclosures. Variability in air flow caused some problems in previous experiments. This variability was primarily due to the poor quality of the needle valves used. These old valves were replaced in this experiment with more accurate microadjusting valves. The new valves were also used in all subsequent experiments. Part of the variability in air flow rates was also a result of measuring air flow at the surface. It was found that air flow measured at the surface did not accurately represent the flow at the depth of air release which was much lower due to hydrostatic pressure. The air flow also varied due to kinks in the air lines and small differences in the release depth. To resolve these problems an in-line flow meter was constructed. The meter, which was used in this and all subsequent experiments, was designed to measure the air flow at the point of release. The air flow rate of 100 ml/min at the surface, used in previous experiments, was found to be equivalent to a flow rate of 50 ml/min at seven meters. This air flow rate was used in all mixed enclosures. All treatments were randomly assigned to enclosures. A summary of these treatments is given in Table 11. Routine measurements and analyses were the same as those described in Experiment 1. Integrated water samples were taken to a depth of 5.5 meters. Zooplankton tows were taken initially in the lake and in all of the closed bottom bags. Final tows were taken in all bags and in the lake. All bags were also screened initially to remove fish.

Changes in pH, CO2, TP, SD, Chla, and algal volume in each enclosure are shown in Figure 31. The pH dropped to a low of 7.1 within six days in both closed bottom bags which received CO2 additions (bags 3 and 6). This was slightly less than the low of 7.3 observed in the open bottom mixed enclosure (bag 1). The pH returned gradually in bag 6 and quickly in bags 1 and 3 to a level near that of the other bags. A similar drop in pH was not observed in the lake, the open control bag (bag 2) or in the other closed bottom bags However, the pH levels in all bags except the closed control (bag 5) were initially quite low and the "high pH" treatment was only slightly different from the "low pH treatment". The initial pH in the closed control (bag 5), was quite high (9). TP increased to 1415 ug/l and 1205 ug/l in the closed bottom enclosures which received nutrient additions (4 and 6 respectively) exceeding the maximum value of 1060 ug/l observed in the open mixed bag (1). The TP level remained high in bags 4 and 6 but declined rapidly in bag 1. This rapid decline of TP in the open bottom bag may represent a loss of particulate phosphorus by sedimentation. Particulate phosphorus could not drop out of the closed bottom bags and was probably resuspended in the water column. The levels of TP in the closed bottom enclosures which did not receive nutrients (bags 3, 5, 7) were similar to those in the lake and open

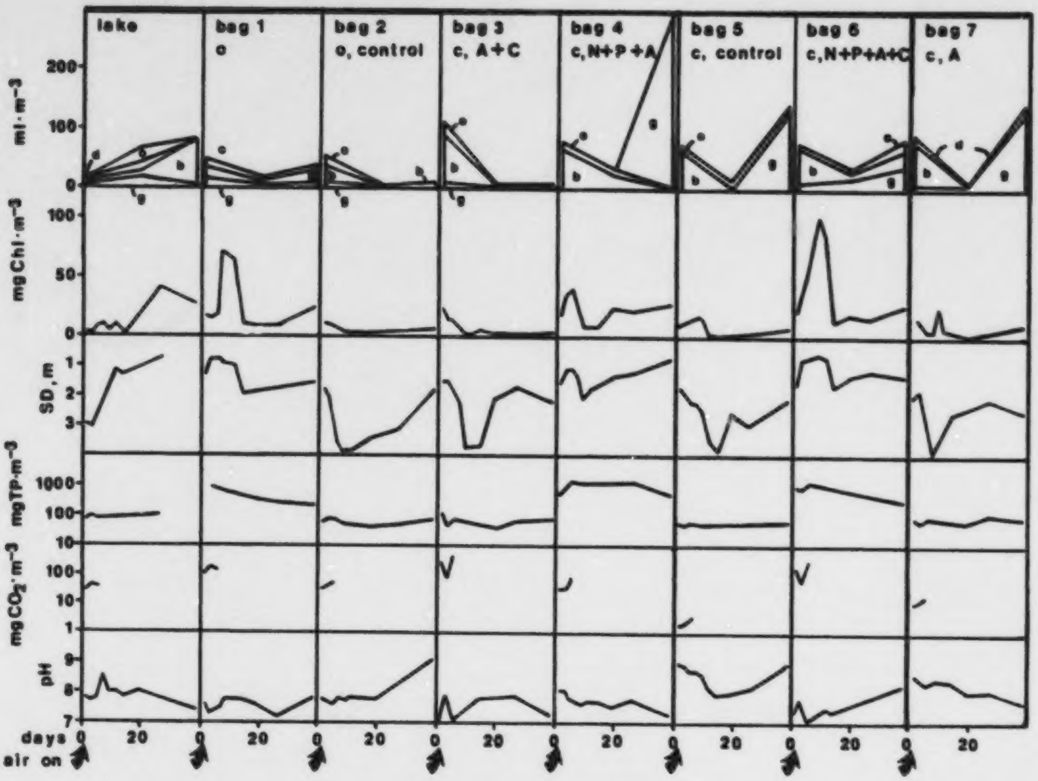


Fig. 31. Changes in pH, CO_2, TP, Secchi disc transparency, chlorophyll a and algal volume in the enclosures and lake during experiment 5. (algal volume: g = greens, b = blue-greens, d = diatoms, o = other; treatment designations: o = open bottom, c = closed bottom, N = nitrogen, P = phosphorus, A = alkalinity, C = CO_2.)

control (bag 2).

The response of the phytoplankton in this experiment was not entirely consistent with our theoretical framework. Oscillatoria spp. dominated in all of the enclosures initially. A definite shift to green algae occurred in four out of the five closed bottom enclosures (4, 5, 6, 7). The pH was not consistently low in these enclosures but it did drop below pH 8 in all of them at least once during the experiment. Since it is not clear whether continued low pH or just a sudden drop is required to produce the blue-green to green shift these results are somewhat ambiguous. High levels of available nutrients, TP/Chl, which are also apparently required for the blue-green to green shift (Shapiro 1973), were observed in all of these enclosures just before the greens bloomed. It is important to note that the blue-green to green shift did not occur in the open bottom enclosures (1 and 2) even though the levels of pH and TP were similar to those in the closed bottom bags. This result seems to indicate that some unknown factor necessary to maintain blue-green dominance was excluded from the closed bottom bags. This factor might have a stimulatory effect on blue-greens, an inhibitory effect on greens or both. The community level response was consistent with previous results. Chl$\underline{a}$ levels were again correlated with TP concentrations. The relationship between average TP and Chl$\underline{a}$ concentrations is shown in Figure 32. This regression is significant at the .05 level. The highest chlorophyll levels were found in the open mixed bag (1) and the two closed bottom enclosures which received nutrient additions (4 and 6). As in previous experiments the stimulatory effect of nutrient enrichment was apparently more important than the negative influence of light limitation when the mixed depth was increased.

The results of the factorial experiment were inconclusive. An analysis of variance was performed to determine the effects of pH and TP on three different dependent variables: The average Chl$\underline{a}$ concentration, the volume of blue-green algae on day 19 and the volume of green algae on day 19. There was no significant effect of pH on any of these variables ($p = .05$). This is not surprising considering the small difference between high and low pH treatments. TP also had no significant effect on these variables. An effect of TP on Chl$\underline{a}$ was expected since a significant linear relationship was already demonstrated between average TP and Chl$\underline{a}$ (Fig. 32). The failure to find a significant effect was primarily due to the small number of degrees of freedom available for the analysis of variance.

Changes in the abundance of herbivorous zooplankton observed in the enclosures are shown in Figure 22. The sample from the initial lake tow was lost. Therefore it is not possible to determine whether a change in herbivore numbers occurred in the lake or open bottom enclosures. However, there was a consistent increase in herbivore numbers in all of the closed bottom bags. The smallest increase occurred in the control bag which had the highest pH levels, as predicted in the theoretical framework.

Experiment 6

Experiment 6 was set up on Twin Lake and lasted from August 5 to September 9, 1977. This was the largest and most ambitious experiment undertaken using 16 enclosures suspended from four interconnected outriggers. The previous experiments indicated that changes in pH and nutrient levels during artificial circulation might be important factors in the phytoplankton response. It was apparent after the first three experiments that the effects of these factors could not be entirely separated in a natural system where many other variables also influence the phytoplankton. As a result, the

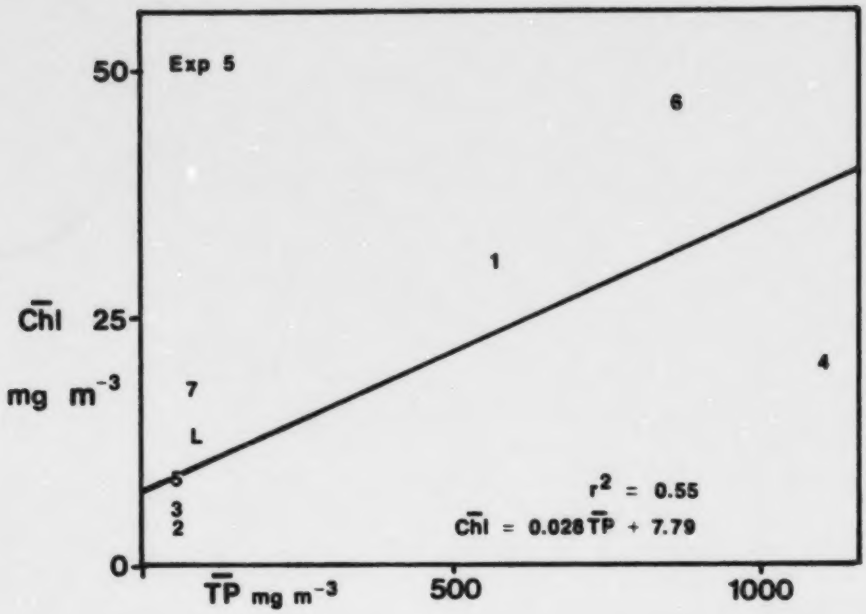


Fig. 32. Relationship between average concentrations of TP and chlorophyll a in the enclosures and lake during experiment 5 (numbers refer to different enclosures, L = lake).

relationships observed in these experiments were generally ambiguous. Subsequent experiments were designed to separate the different factors as much as possible using closed bottom enclosures and controlled chemical additions. However, the closed bottom bags were only successful in separating different hypolimnetic factors and there were still some uncontrolled factors which made the results difficult to interpret (e.g. zooplankton, light limitation). In experiment six we decided to take a somewhat different approach. Instead of trying to study the effect of each factor separately an attempt was made to investigate the interaction between factors and their combined effect on the phytoplankton. In particular, the experiment was designed to investigate two different interaction mechanisms which might be important during artificial circulation: 1) the interaction between grazing and pH in the blue-green to green shift mechanisms, and 2) the interaction between all of the factors in Equation 11 in the community response mechanism.

The interaction between grazing and pH in the blue-green to green shift mechanism

The results of previous experiments suggested that even when conditions favor a blue-green-green shift (i.e. low pH, high nutrient availability) herbivores may prevent it from occurring by grazing selectively on the green algae (cf. expt. 2, bags 8 and 9). A factorial experiment was designed to test this hypothesis using eight closed bottom enclosures. Two levels of pH and two levels of grazing were used. The pH was varied by hypolimnetic CO2 additions. The abundance of herbivores was reduced in the "low grazing" treatments by adding four planktivorous fish (*Lepomis gibbosus*) to each of these enclosures. Nitrogen, phosphorus and alkalinity were added to all of these to simulate natural hypolimnetic levels and all of these were mixed to seven meters with an air flow rate of 50 ml/minute. Each treatment was duplicated so that interaction effects could be determined and to raise the number of degrees of freedom for the analysis of variance. Mixed, open bottom bags with and without fish were also included for comparison. The general experimental design and enclosure numbers associated with each treatment are indicated below.

	Closed Bottom Enclosures	
	High pH	Low pH
High grazing	9, 2	5, 14
Low grazing	3, 13	11, 8

Open Bottom Enclosures	
High grazing	1, 7
Low Grazing	6, 16

The effect of these treatments on the blue-green-green shift was measured in several ways. The growth rate was determined for individual green and blue-green species. Composite growth rates were also calculated at the division level and the difference between the growth rates of blue-greens and greens was used to indicate competitive advantage. In addition, rates of photosynthesis were determined for a number of blue-green and green species autoradiographically in bags 3, 5, 9 and 11.

The community response mechanism

The second part of the experiment was an investigation of the interaction among all factors in equation 11 during artificial circulation and their combined effect on algal community standing crop. This general mechanism was investigated by 1) evaluating all of the parameters of equation 11 with data from several different enclosures representing a range of mixing conditions, 2) testing the reliability of the model on other enclosures and 3) using the model to simulate the effects of a wide range of mixing conditions on algal standing crop. All of the closed bottom bags in the factorial design (described above) were monitored along with two unmixed control bags (10,15) in order to evaluate the model parameters (Ec and Ew). The evaluation of the remaining parameters was based on measurements restricted to bags 3, 5, 8, 9, 10, and 11. Bag 8 was included after bag 11 developed a leak. All treatments were randomly assigned to enclosures. A complete summary of treatments is given in Table 12. Most of the sampling procedures and analyses used in experiment 6 were the same as those described in experiment 5. However, several modifications and additional procedures were required. Routine integrated samples were taken to a depth of 5.5 meters. Initial and final zooplankton tows were taken in all of the enclosures and in the lake. Photosynthetic rates were measured at both the community and species levels approximately every four days in bags 3, 5, 8, 9, 10 and 11. Seven-meter integrated samples taken for the productivity measurements were also analyzed for pH, Chl *a*, POC, TP, and alkalinity. Light measurements were recorded continuously at a site 5 miles southwest of the lake.

Changes in pH, CO2, TP, SD, Chl *a* and algal volume observed in the top 5 m of each enclosure are indicated in Figure 33.

The results of the factorial analysis of the effects of grazing and pH were inconclusive. The only effects considered in the analysis were those observed during the first 6 days since this was the only period during the experiment where the pH in the "high" and "low" pH treatments differed significantly (cf. Fig. 33). Six-day growth rates were calculated for blue-greens and greens at both the division and species level while species specific rates of photosynthesis were determined on day 6. These values represent the average growth and photosynthetic rates of populations in the mixed layer. The competitive advantage of greens over blue-greens was also calculated from these six-day growth rates. A separate analysis of variance was done for each of these dependent variables. The significance of each main (pH or grazing) and interaction effect was determined with an F-test at the .05 level. A summary of the results of these analyses is presented in Table 13. None of the F-tests were significant at the .05 level. There are several reasons why no significant effects of either grazing or pH were found. The difference between "high" and "low" pH treatments was generally less than 1 pH unit. (cf. Fig. 33). This change was apparently too small to affect the competitive advantage of green over the blue-green algae. As in the previous Twin Lake experiment, this small drop in the pH was primarily due to the high alkalinity and relatively low hypolimnetic CO2 concentrations characteristic of the lake. A similar drop in pH was observed in the open bottom mixed bags. The lack of a significant grazing effect in the analysis was apparently a result of the failure of planktivorous fish in the "low grazing" treatments to remove herbivorous zooplankton. Their general ineffectiveness was indicated by the lack of any significant difference between the final zooplankton counts in enclosures with and without fish (Fig. 22). In addition, the grazing and pH effects may have been confounded by the high

Table 12: Treatments for enclosures in Experiment 6, Twin Lake

Bag	Air on	Air off	Air flow ml min^{-1} @ 7 m	Air Release depth (m)	Bag Type	Additions*	Text designation
1	8/5/77	9/9/77	50	7	open	none	open low pH high grazing
2	"	"	50	7	closed	N+P+A	closed high pH high grazing
3	"	"	50	7	closed	N+P+A+F	closed high pH low grazing
4	--	--	--	--	open	none	open control
5	"	"	50	7	closed	N+P+A+C	closed low pH high grazing
6	"	"	50	7	open	F	open low pH low grazing
7	"	"	50	7	open	none	open low pH high grazing
8	"	"	50	7	closed	N+P+A+C+F	closed low pH low grazing
9	"	"	50	7	closed	N+P+A	closed high pH high grazing
10	--	--	--	--	closed	none	closed control
11	"	"	50	7	closed	N+P+A+C+F	closed low pH low grazing
12	"	"	50	7	open	none	open control
13	"	"	50	7	closed	N+P+A+F	closed high pH low grazing
14	"	"	50	7	closed	N+P+A+C	closed low pH high grazing
15	--	--	--	--	closed	none	closed control
16	"	"	50	7	open	F	open low pH low grazing

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*A(Alkalinity, $NaHCO_3$), N(Nitrogen, NH_4Cl), P(Phosphorus, K_2HPO_4), C(CO_2), F(Fish).

Table 13: Factorial analysis for the effects of pH and grazing on algal growth and photosynthesis in Experiment 6.

	Treatment	high pH high graz.		high pH low graz.		low pH hign graz.		low pH low graz.		Test of Significance* at .05 level Effects		
Dependent var.	Bag	9	2	3	13	5	14	11	8	pH	graz.	inter.
r chla		.2870	.2780	.2423	.2450	.1989	.2384	m	.2615	-	-	+
r phytoplankton		.0569	.1696	.2560	.1117	.1800	.0984	m	.2062	-	-	-
r greens		.0306	.0970	.1197	.1715	.0376	-.0396	m	.0936	-	-	-
r blue greens		.0087	.1710	.2084	.1487	.1813	.0999	m	.1579	-	-	-
r diatoms		.2587	.2750	.3989	.4421	.5101	.4464	m	.2805	-	-	+
r d-b		.2500	.1040	.1905	.2934	.3288	.3465	m	.1226	-	-	-
r b-g		.0393	.0740	.0887	-.0228	.1437	.1395	m	.0643	-	-	-
r d-g		.2893	.1780	.2792	.2706	.4725	.4860	m	.1869	-	+	+
r Oocystis		.0400	-.0330	.3119	.0900	.1846	.1500	m	.2871	-	-	-
r Sphaerocystis		.0511	-.0350	.0823	.1775	.1563	-.1340	m	-.0280	-	-	-
r bg coccoids		.1474	.0931	.2593	.1305	.3240	-.1819	m	.0736	-	-	-
r monad		-.2897	-.0980	.2735	-.2094	-.1017	m	m	.1881	-	-	-
r Cryptomonas		.0028	.2030	.2640	.2506	.1987	.0004	m	.1795	-	-	-
r Melosira		.3563	.3690	.5530	.3090	.4934	.1969	m	.2912	-	-	-
r Fragilaria		.1428	.1810	.2200	.3925	.5534	.3041	m	.2764	-	-	-
r Oscillatoria		.0439	.2380	.0692	.1404	.2330	.0625	m	.2366	-	-	-
r Anabaena		.2130	.1950	.2630	.0852	.1761	.1102	m	.0340	-	-	-
r Synedra		.2035	.2730	.0578	-.0492	.2936	-.3900	m	.0578	-	-	-
r Aphanizomenon		.0286	.1446	.1923	.1607	.1315	.0639	m	.2202	-	-	-
p Anab. (gC cell^{-1} d^{-1})		182.7		30.8		41.8		36.0		-	-	
p Aphan. (gC 100um^{-1} d^{-1})		1404.0		213.2		245.3		391.8		-	-	
P Frag. (gC cell^{-1} d^{-1})		68.3		29.5		47.8		85.2		-	-	
p Crypt (gC cell^{-1} d^{-1})		256.0		267.7		202.6		246.6		-	-	
p Mel. (gC 100um^{-1} d^{-1})		247.1		112.9		130.1		294.0		-	-	
p Sphaer. (gC cell^{-1} d^{-1})		74.1		28.0		23.3		34.3		-	-	

* + = yes, - = no

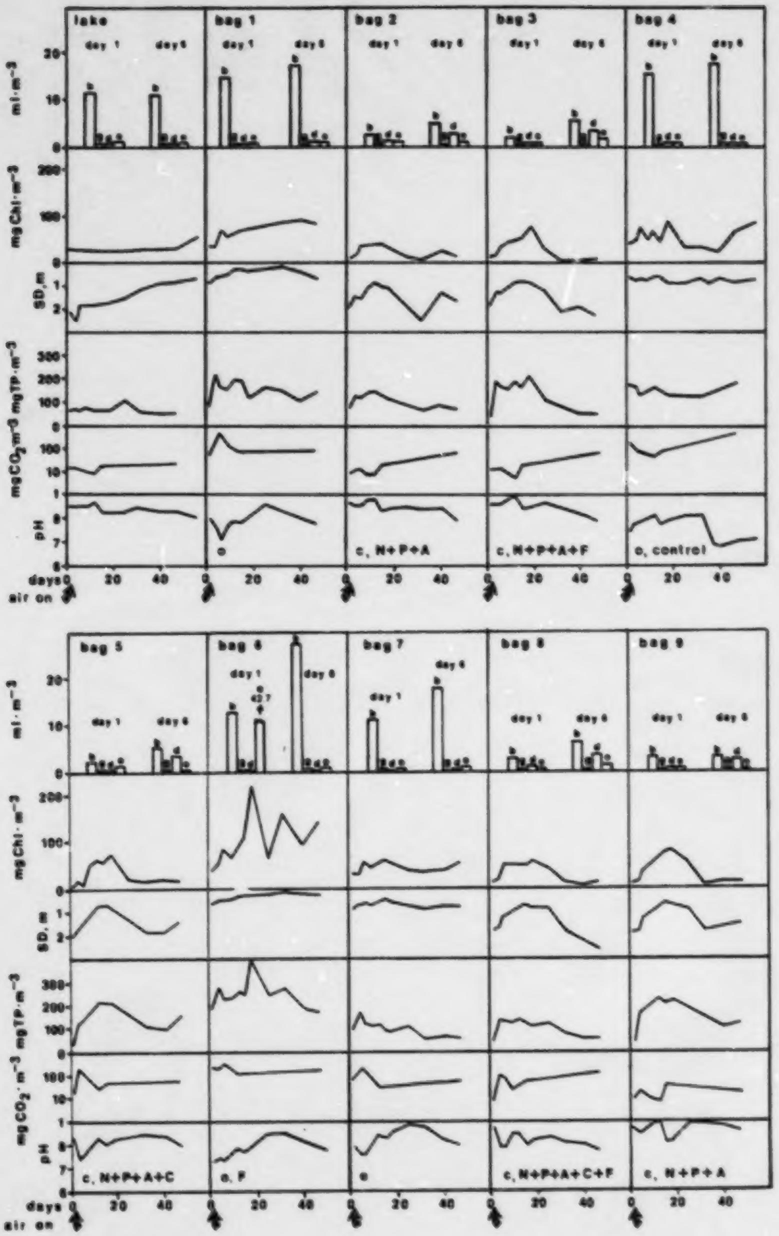

Fig. 33. Changes in pH, CO_2, TP, Secchi disc transparency, chlorophyll a and algal volume in the enclosures and lake during experiment 6. (algal volume: g = greens, b = blue-greens, d = diatoms, o = other; treatment designations: o = open bottom, c = closed bottom, N = nitrogen, P = phosphorus, A = alkalinity, C = CO_2, F = fish)

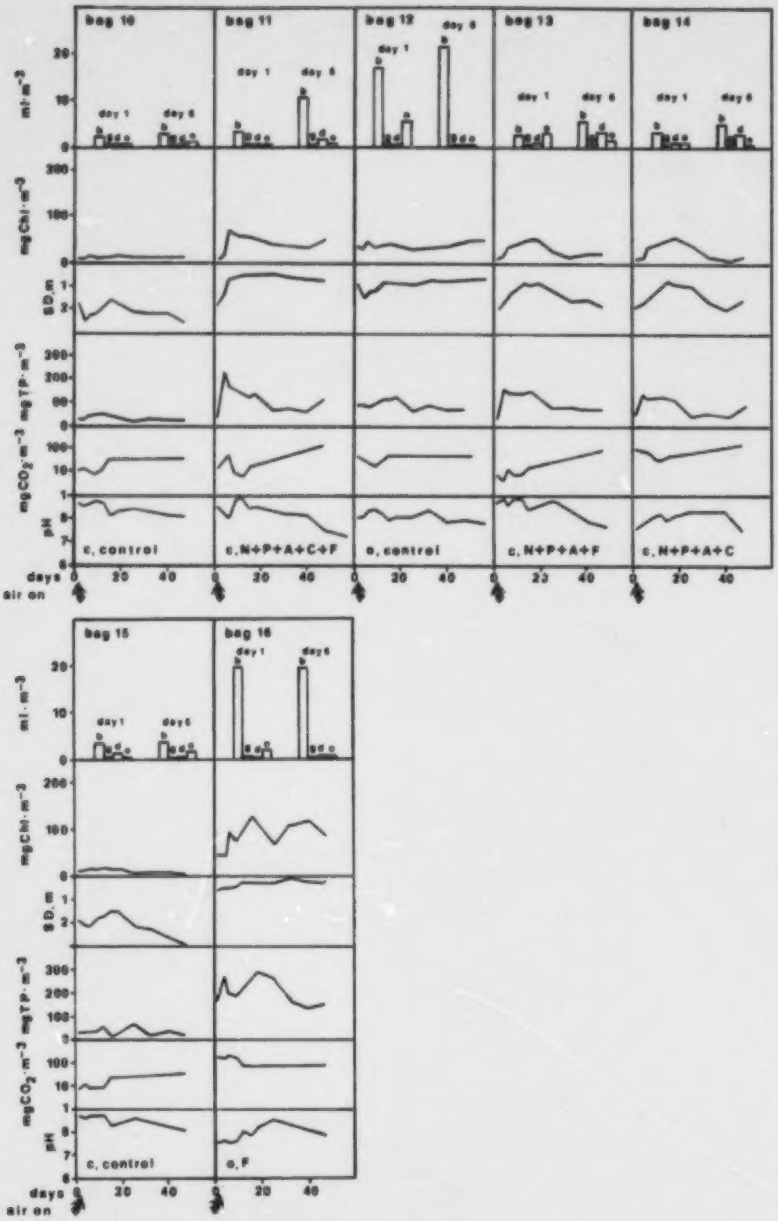

Fig. 33 Cont'd. Changes in pH, CO_2, TP, Secchi disc transparency, chlorophyll a and algal volume in the enclosures and lake during experiment 6. (algal volume: g = greens, b = blue-greens, d = diatoms, o = other; treatment designations: o = open bottom, c = closed bottom, N = nitrogen, P = phosphorus, A = alkalinity, C = CO_2, F = fish)

variability in nutrient levels observed in the closed bottom bags (Fig. 33).

The analysis of community response mechanisms began with an evaluation of several factors in equation 11 which were relatively constant. The extinction coefficient of chlorophyll, Ec, and the residual extinction coefficient of the water, Ew, were determined from a regression of the total extinction coefficient, E , on the concentration of chlorophyll a. The total extinction coefficient was determined from Secchi disc readings using the Lambert-Bouguer Law and assuming that the light intensity at the Secchi depth, Isd, was 15% of the surface intensity, Io (Megard, personal communication).

$$Et = \frac{\ln(Io/Isd)}{SD} = \frac{1.897}{SD} \qquad (16)$$

The regression is shown in Figure 34. The slope of the regression line, 0.023 m-2 mg Chl-1, is an estimate of Ec. The y-intercept, 0.78 m-1, is an estimate of Ew. The light limitation factor, ln (Io/Iz'), is normally calculated from the maximum daily volumetric rate of photosynthesis, p-max, the integral rate of photosynthesis, π, and the total extinction coefficient, E , as

$$\ln(Io/Iz') = \frac{\pi\, Et}{p\text{-max}} \qquad (17)$$

Unfortunately, ln (Io/Iz') could not be calculated from our data because p-opt was not measured directly. Megard et al. (1979) reported an average ln (Io/Iz') value of 1.9±0.18 for populations in Halstead's Bay, Minnesota. Megard et al. (1978) also showed that the value of ln (Io/Iz') was relatively constant (1.9-2.0) when daily incident irradiance exceeded 320 Langleys day-1). Since daily incident irradiance was greater than 380 Langleys day-1 for all incubations in experiment 6 we have assumed a constant value of 1.9 for ln (Io/Iz') in the present analysis. The nutrient saturated maximum daily specific rate of photosynthesis, P-max, and the subsistence quota of phosphorus, Kq, were determined from a regression of the maximum daily specific rate of photosynthesis, P-max, on the ratio c/TP. P-max was not measured directly but was calculated as:

$$P\text{-max} = \frac{\pi\, Et}{1.9c} \qquad (18)$$

Only data from the first 17 days were included in this regression. Periphyton growth on the sidewalls of the enclosures became a significant problem past this point and the photosynthesis results were inconsistent. The regression (shown in Fig. 35) was significant at the .05 level and represents the linear transform of equation 7:

$$P\text{-max} = P\text{-}{}^{sat}_{max}\; Kq\; \frac{c}{TP} + P\text{-}{}^{sat}_{max} \qquad (19)$$

The y-intercept, 140 mg Cmg Chl-1 d-1, is an estimate of P-max, and the

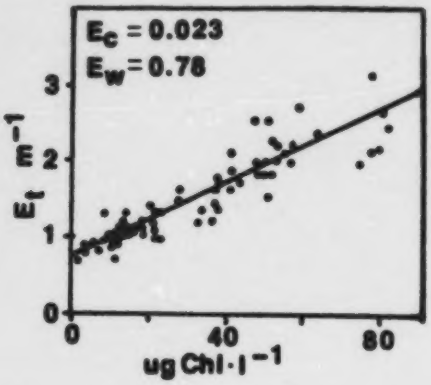

Fig. 34. Relationship between the total extinction coefficient and chlorophyll a in the closed bottom enclosures during experiment 6.

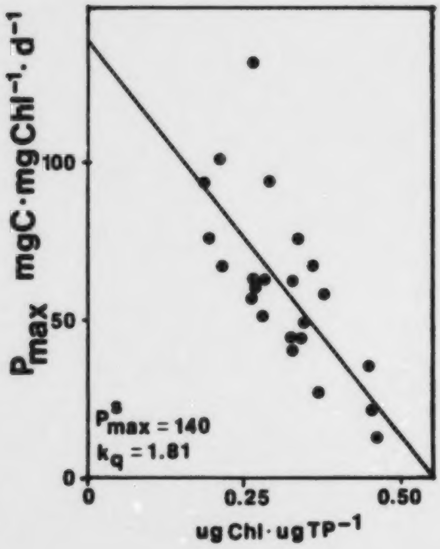

Fig. 35. Relationship between the maximum daily specific rate of photosynthesis and the ratio chlorophyll a/TP in several of the closed bottom enclosures during the first 20 days of experiment 6.

inverse of the x-intercept, 1.81 mg TP mg Chl-1, is an estimate of Kq. The carbon to chlorophyll ratio in the algae, Θ, was determined from a regression of the concentration of particulate organic carbon on the concentration of chlorophyll measured in the enclosures (Fig. 36). The slope of this regression line, 41 mg C mg Chl-1, is an estimate of Θ. The finite specific loss rate, D, was determined at frequent intervals in the enclosures where photosynthesis was measured. It was calculated as the difference between the finite specific growth rate and the finite specific rate of carbon fixation (see the Methods section for a more detailed discussion of this calculation). The average value of D determined during the experiment was 0.110 ± 0.032 day-1. The two remaining parameters in equation 11, TP and Zm, were considered variables in the analysis. The equilibrium model (equation 11) and a summary of parameter values are given below:

$$C^* = \frac{\ln(Io/Iz')\,P\text{-}^{sat}_{max} - D\Theta ZmEw}{EcD\Theta Zm + (\ln(Io/Iz')\,P\text{-}^{sat}_{max}\,Kq)/TP} \qquad (20)$$

P-max = 140 mg C mg Chl-1 d-1
Kq = 1.81 mg TP mg Chl-1
Ec = 0.023 m-2 mg Chl-1
Ew = 0.78 m-1
ln (Io/Iz') = 1.9
D = 0.110

The reliability of the model was tested by comparing the equilibrium concentration of chlorophyll predicted by the model to the actual chlorophyll concentration observed at the point of maximum yield (C/TP) in each closed bottom enclosure. The TP concentration at the point of maximum yield and mixed depth in each enclosure were used in the model prediction. All closed bottom enclosures except bag 11 (which leaked) were considered in the analysis, including those which were not involved in the evaluation of P-max, Kq, and Θ.

The relationship between predicted and observed equilibrium chlorophyll concentrations is shown in Figure 37. If the agreement between observed and predicted results was perfect all points would fall on a line with slope equal to 1 and which intersects the origin (shown by the dashed line in Fig. 37). The slope of the actual regression line (not shown) was not significantly different from 1 and the y-intercept was not significantly different from 0. There was also a linear relationship between equilibrium chlorophyll and TP concentrations (Fig. 38). Both predicted and observed equilibrium chlorophyll concentrations showed linear relationships to TP and the slopes and intercepts were not significantly different at the .05 level. As in previous experiments, a similar relationship was also found between average concentrations of TP and Chl a in all of the enclosures in experiment 6 (Fig. 39). These results indicate that the mathematical model presented here provides a consistent theoretical framework for addressing community response patterns.

After these preliminary tests the model was used to simulate the relationship between maximum chlorophyll concentrations, c* and the mixed depth at several levels of TP. The results are shown in Figure 40a. The TP values chosen were those observed in several of the enclosures and the black circles represent the observed results. The model predicts that, at a single level of TP, the maximum chlorophyll concentration will decrease as the mixed

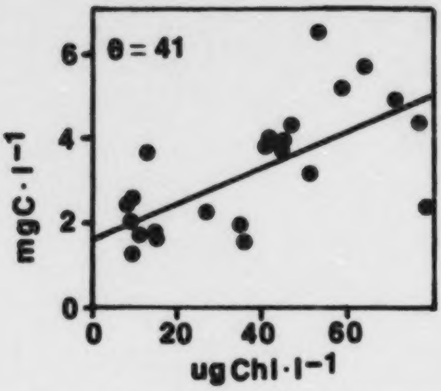

Fig. 36. Relationship between the concentrations of chlorophyll a and particulate organic carbon in several closed bottom enclosures during experiment 6.

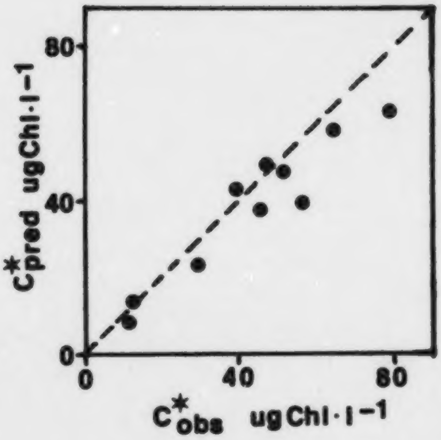

Fig. 37. The agreement between the maximum observed concentration of chlorophyll a and that predicted by the model in the closed bottom enclosures and in the lake during experiment 6 (the dotted line represents perfect agreement).

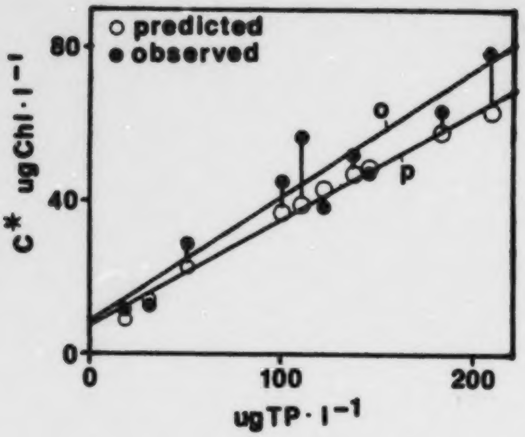

Fig. 38. The relationship between TP and the maximum concentration of chlorophyll a in the closed bottom bags and in the lake during experiment 6. Both predicted (open circles) and observed (closed circles) results are shown along with their associated regression lines.

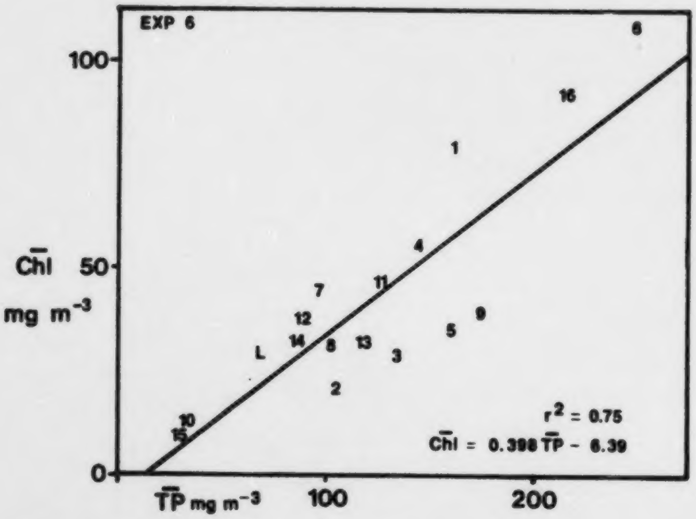

Fig. 39. The relationship between average concentrations of TP and chlorophyll a in the enclosures and lake during experiment 6 (numbers refer to different enclosures, L = lake).

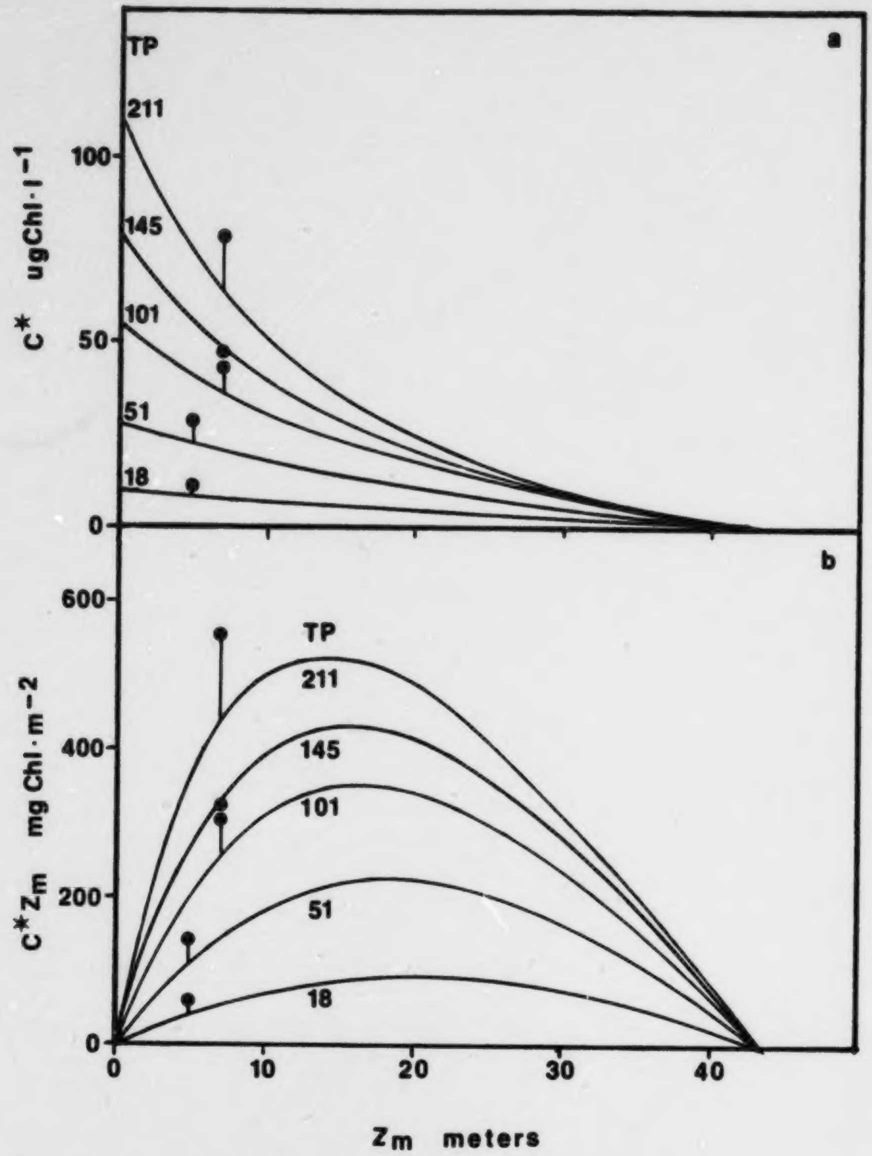

Fig. 40. The effect of changes in the mixed depth and TP on (top) (a) the maximum concentration of chlorophyll a and (bottom) (b) the maximum aerial standing crop of chlorophyll a in the mixed layer of Twin Lake, as predicted by the model (closed circles indicate observed results; short lines connecting circles with the simulation lines indicate the deviation between predicted and observed results).

depth is increased due primarily to increased light limitation. However, if the concentration of TP rises during mixing the maximum chlorophyll concentration may increase, as it did in the experimental enclosures in spite of the light limitation effect. If the TP increases from 18 to 100 ug Pl-1 during mixing, the mixed depth would have to become greater than 30 meters for a reduction in c* to occur.

The effect of changes in Zm and TP on maximum total algal standing crop (C*Zm) was also simulated and the results are shown in Figure 40b. Again the black circles represent values observed in the experimental enclosures. The model predicts that at a given TP concentration, the maximum total standing crop will increase with the mixed depth to a depth of about 15 meters and then decline steadily to a depth of 43 meters below which the community is eliminated. As with c*, an increase in TP will always result in a higher value of c*Zm than would otherwise be expected. This is especially true at relatively shallow mixed depths such as those used in experiment 11. C*Zm increased dramatically after the mixed depth was increased from 5 to 7 meters in the experimental enclosures due primarily to an increase in TP.

Although D was assumed to be constant in this analysis there was some indication that differences in herbivorous grazing between bags had a significant effect on the yield of chlorophyll. As in experiment 4, a linear relationship was found between TP/Chl a (inverse of yield) and community grazing rates determined at the end of the experiment (Fig. 41). The lower yield associated with the highest grazing rates suggests that D was significantly higher in these bags.

Experiment 7

There was evidence in several of the previous experiments that rapid mixing can occasionally inhibit algal growth or reduce the yield of chlorophyll. This effect was most obvious in the open bottom enclosures (for example bag 7 in experiment 1 and bag 10 in experiment 4). These results led us to conclude that we may have overlooked some important effects of artificial circulation by using closed bottom enclosures and restricting our air flow rates to relatively low levels. The last two experiments were designed to take another look at the effect of mixing rate on algae. Only open bottom bags were used and the fastest mixing rates were 5 to 6 times greater than those commonly used in the previous experiments.

Experiment 7 was set up on Little Lake Johanna and lasted from July 6 to July 26, 1978. The experiment was primarily designed to determine the mixing rate required to produce the "inhibition effect" which was often observed in rapidly mixed open bottom bags. The design therefore consisted of a broad range of mixing rates (0-300 ml/min. @ 7m in open bottom bags). The effect of "light limitation" was also investigated. An attempt was made to simulate the degree of light limitation expected in an exceptionally deep lake following destratification. To accomplish this two of the enclosures (13 and 14) were constructed of a combination of black and clear polyethyelene. The top meter of both enclosures was clear and the lower 7 meters was black. This had the effect of reducing the proportion of the population within the euphotic zone. A complete list of treatments is given in Table 14.

Integrated samples were taken to a depth of 4 meters in this experiment. All other sampling and analytic procedures were the same as in experiment 1. Changes in pH, TP, SD, and Chl a in each enclosure are shown in Figure 42. The pH was relatively low in all enclosures during the experiment. TP was generally higher in the mixed bags but was not correlated to Chl a as it was in previous experiments. Figure 43 shows the relationship between average TP

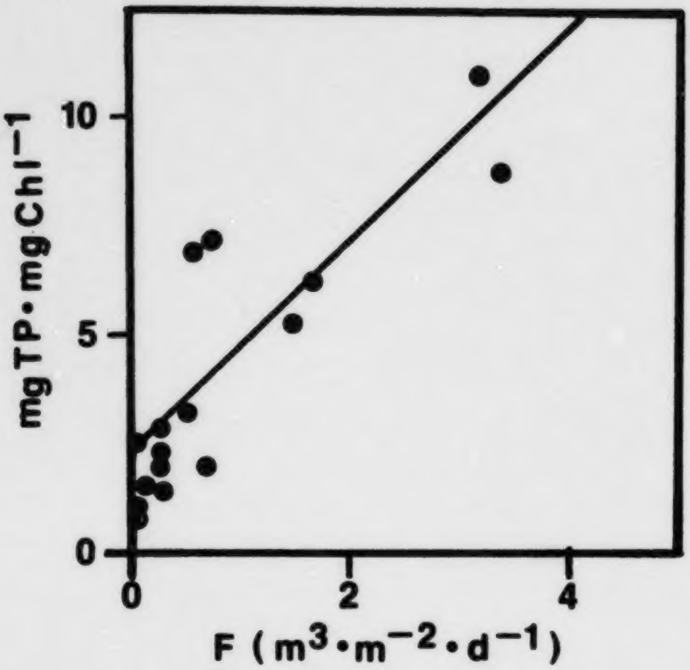

Fig. 41. Relationship between the total herbivore filtration rate (F) and the ratio of TP/chlorophyll a in the enclosures and lake on the final day of experiment 6.

Table 14: Treatments for enclosures in Experiment 7, Little Lake Johanna

Bag	Air on	Air off	Air flow (ml min @ 7 m)	Air release depth (m)	Bag type	Text designation
1	7/6/78	7/26/78	10	7	open	10
2	"	"	20	7	open	20
3	"	"	50	7	open	50
4	"	"	75	7	open	75
5	"	"	100	7	open	100
6	"	"	125	7	open	125
7	"	"	150	7	open	150
8	"	"	175	7	open	175
9	"	"	200	7	open	200
10	--	--	0	--	open	Control
11	"	"	300	7	open	300
12	--	--	0	--	open	Control
13	"	"	50	7	open w/black	50 black (7m black/1m clear)
14	"	"	75	7	open w/black	75 black (7m black/1m clear)

Table 15: Treatments for enclosures in Experiment 8, Twin Lake

Bag	Air on	Air off	Air flow (ml min @ 7 m)	Air release depth (m)	Bag type	Text designation
1	8/11/78	9/13/78	250	7	0	250
2	--	--	0	--	0	Control
3	8/11/78	9/13/78	250	7	0	250
4	--	--	0	--	0	Control

Table 16: Herbivore grazing rates obtained from the literature or calculated from a regression of literature values.*

Species	mm $\bar{L}$	ml/animal/dy F	Source
Daphnia rosea	1.45	4.6	Haney 1973, Burns & Rigler 1967
D. galeata	1.60	5.1	Haney 1973, Burns & Rigler 1967
D. parvula	0.95	3.8	Haney 1973
D. longispina		2.3	Nauwerck 1963
D. retrocurva	0.90 (carapace)	2.8	Regression*
D. pulex	1.75	5.4	Regression*
D. ambigua	0.88	2.7	Regression*
Ceriodaphnia quadrangula	0.80	4.6	Haney 1973
Diaphanosoma brachyurum	1.15	1.6	Haney 1973
Bosmina longirostris	0.50	0.4	Haney 1973
Chydorus sphaericus	0.15	0.2	Haney 1973
Camptocerus rectirostris	1.00	3.1	Regression*
Pleuroxus procurvus	0.50	1.5	Regression*
Scapholebris Kingii	0.90	2.8	Regression*
Simocephalis vetulus	3.00(max)	9.4	Regression*
S. serrulatus	2.90	9.1	Regression*
Diaptomus siciloides		2.0	Comita 1964

Regression for Cladocerans

$F = 3.16\,\bar{L} - .09.$ $r^2 = .60$ $n = 8$

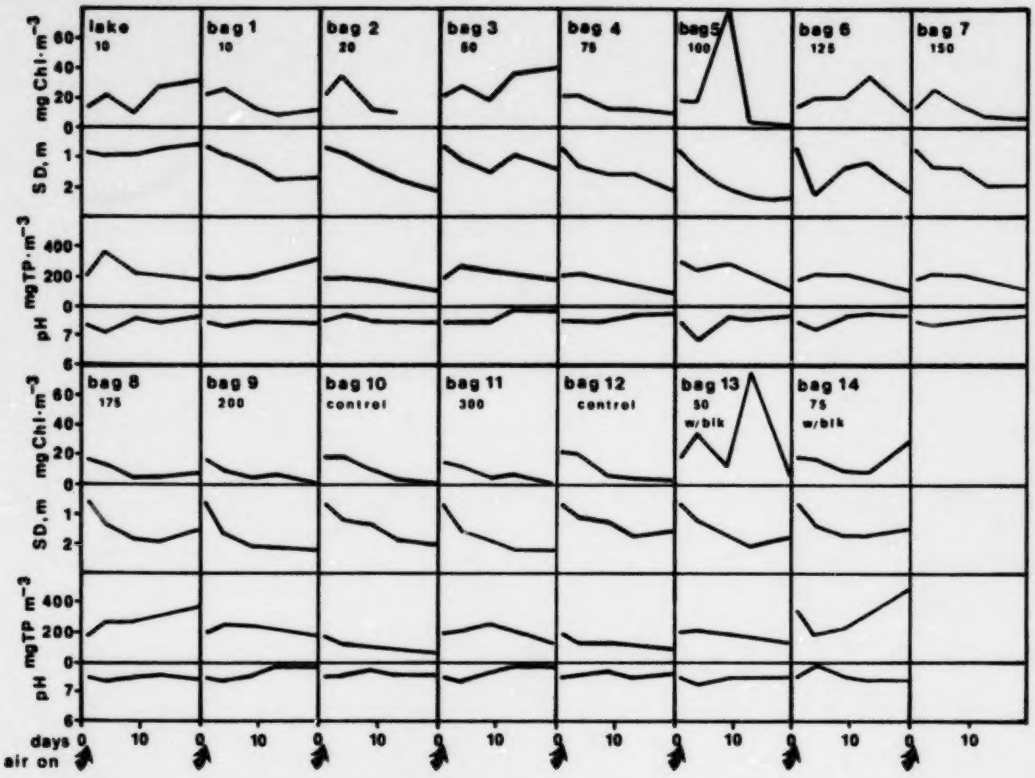

Fig. 42. Changes in pH, TP, Secchi disc transparency and chlorophyll a in the enclosures and lake during experiment 7 (w/blk indicates enclosures utilizing black plastic).

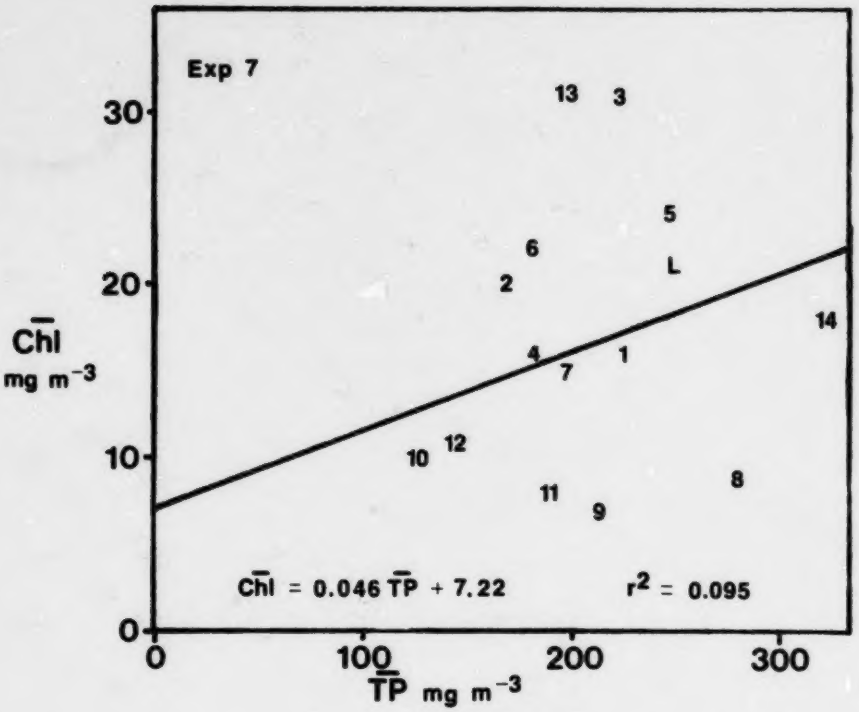

Fig. 43. Relationship between average concentrations of TP and chlorophyll a in the enclosures and lake during experiment 7 (numbers refer to different enclosures, L = lake).

and chlorophyll concentrations in the enlcosures. The regression is not significant. It is important to note that the lowest yield (Chl/TP) occurred in bags 8, 9, and 11, which had the fastest mixing rates. The correlation becomes significant if these points are omitted. This provides additional support for the hypothesis that some unknown factor associated with rapid mixing can inhibit the algae. The factors most likely to be involved in this inhibition effect will be considered in the discussion section. The species composition changed dramatically in several of the enclosures. All enclosures were initially dominated by the blue-green alga, Anabaena circinalis. The composition in most of the bags switched to predominantly diatom and green species by the end of the experiment. The switch occurred first in the rapidly mixed bags and then eventually occurred in 11 out of the 14 bags (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14) including both controls. A similar shift did not occur in the lake. The abundance of herbivorous zooplankton increased in all enclosures except bag 12 (control) during the experiment (Fig. 22). The fact that this increase was observed under all treatment conditions as well as in the lake indicates that some uncontrolled factor common to all of the bags was responsible. This factor may have been pH which was low in all of the enclosures during the experiment. Our theoretical framework suggests that lower pH should be favorable to the development of herbivorous zooplankton.

Experiment 8

Experiment 8 was set up on Twin Lake and lasted from August 11 to September 13, 1978. Four open bottom enclosures were suspended from a single raft. The experiment was designed to determine whether the mixing rates which inhibited algae in experiment 7 would have a similar effect on Twin Lake algae. Only one mixing rate (250 ml/min. @ 7m) was tested. Treatment assignments are listed in Table 15.

Integrated samples were taken to a depth of 5.5 meters. Zooplankton tows were taken three times during the experiment. All other procedures were the same as in experiment 1. Changes in pH, TP, SD, and Chl a are indicated in Figure 44. The pH dropped initially about 1 unit in both rapid mix enclosures (1 and 3) while the pH increased in the control bags (2 and 4). As in previous experiments TP increased in the mixed bags and TP was highly correlated with Chl a. Figure 45 shows the relationship between average TP and Chl concentrations in the enclosures. The regression is highly significant and similar to those determined in previous Twin Lake experiments (experiments 3 and 6). No inhibition was evident as was found in experiment 7. All enclosures were dominated by Oscillatoria rubescens throughout the experiment. Initial zooplankton samples were lost. However, the abundance of herbivorous zooplankton was higher in the mixed enclosures than in the controls and the lake on the other two sampling dates (see Fig. 22). Circulation apparently had a stimulatory effect on the zooplankton as predicted in the theoretical framework.

DISCUSSION

The experimental results presented in this section provide evidence for the action of a number of specific response mechanisms affecting algal species composition and abundance during artificial circulation.

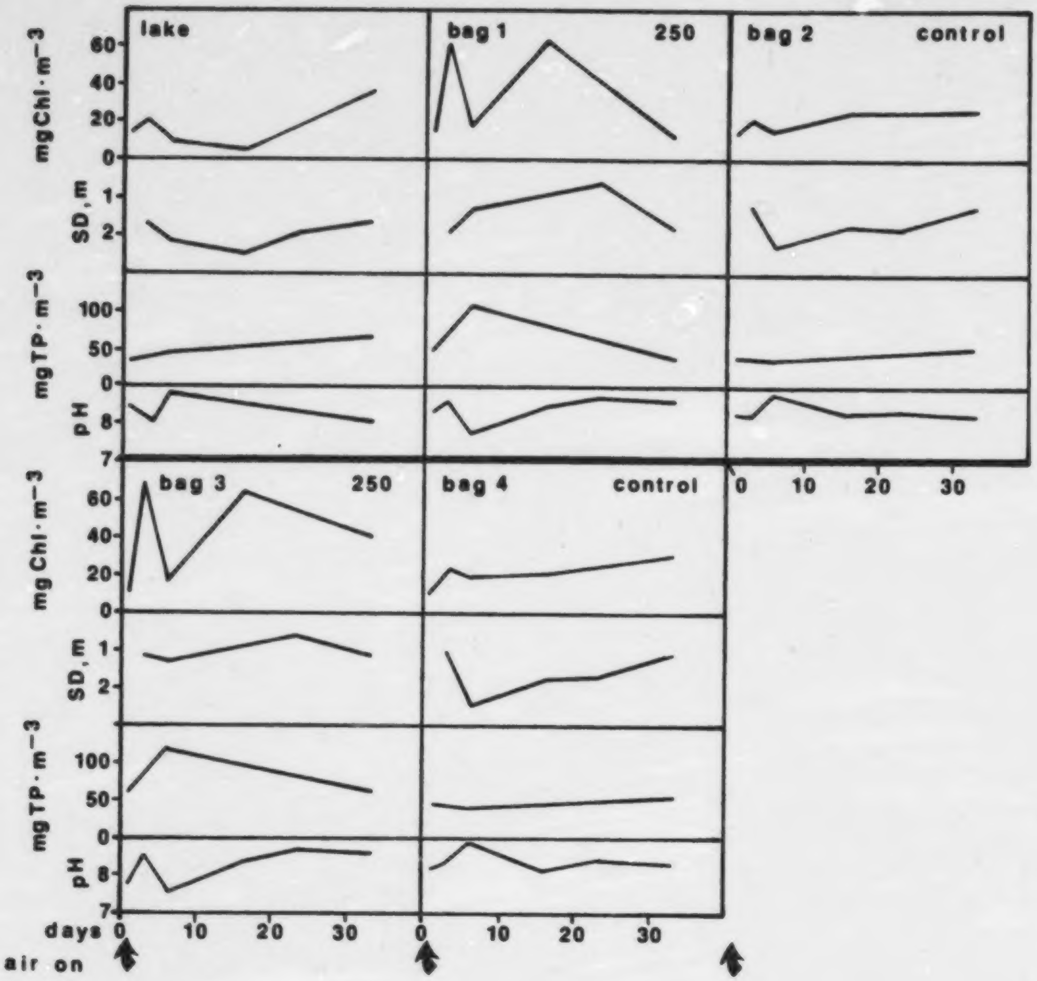

Fig. 44. Changes in pH, TP, Secchi disc transparency and chlorophyll a in the enclosures and lake during experiment 8.

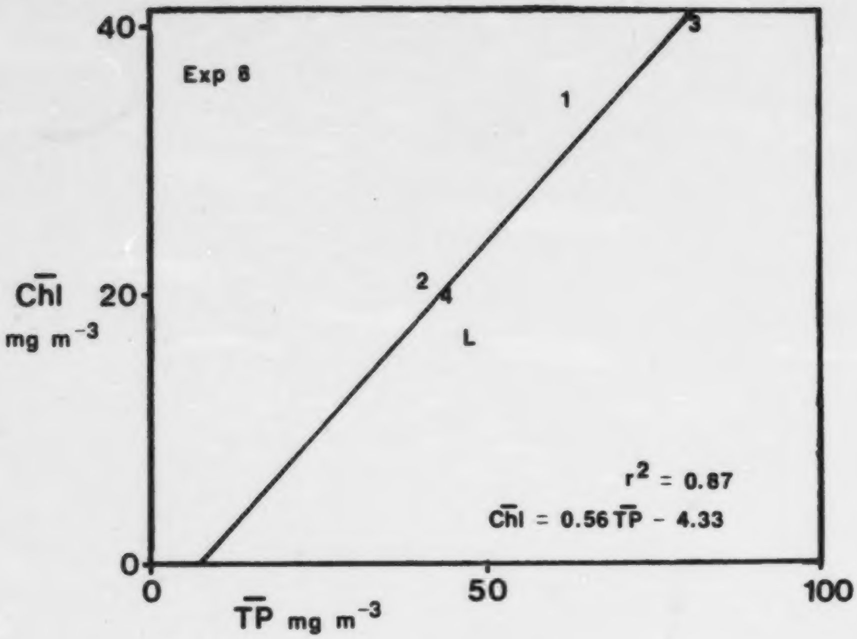

Fig. 45. Relationship between average concentrations of TP and chlorophyll a in the enclosures and lake during experiment 8 (numbers refer to different enclosures, L = lake).

Response Mechanisms Affecting Species Composition

The response of the phytoplankton at the species level seemed to depend primarily on changes in water chemistry which occurred in the euphotic zone during mixing. These changes in water chemistry depended, in turn, on the mixing rate used and on individual lake chemistry. This was most clearly demonstrated in the open bottom bags of experiments 1, 2 and 3. Rapid mixing generally resulted in high concentrations of CO2 and nutrients at the surface accompanied by reduced pH levels. These conditions seemed to favor the development of green algae and diatoms while suppressing the growth of blue-green algae (cf. experiments 1 and 2). This shift to greens and diatoms occurred most frequently in rapidly mixed Little Lake Johanna enclosures. This was apparently a result of the lower pH and higher nutrient levels achieved during mixing due to lower alkalinity and higher hypolimnetic levels of CO2 and nutrients. Nutrient concentrations also increased at the slower mixing rates. However, these mixing rates were apparently insufficient to produce a significant increase in CO2 at the surface and therefore, the pH usually remained high. These conditions of both high pH and high nutrient levels seemed to favor the development of blue-green algae. The blue-green, Microcystis aeruginosa grew particularly well under these conditions reaching high densities in both of the slow mixed enclosures in experiment 2. The shift in competitive advantage from blue-greens to greens which was observed as the mixing rate was increased was apparently related to changes in pH (see Fig. 21). While we were able to isolate the effects of pH and nutrients from other hypolimnetic factors by using closed bottom bags in experiments 4, 5 and 6, the details of this pH-shift mechanism are still unclear. There was some indication that the shift to greens at low pH may have been due to the selective inhibition of blue-green algae. This can be seen in the results for experiment 1 (Fig. 21) where the growth rates for blue-greens decreased steadily as the pH dropped in the deep mixed enclosures. Shapiro (1973) also found that blue-greens were suppressed at low pH in his field experiments. The mechanism for this suppression may involve the activity of cyanophage. Lindmark (1979) demonstrated a dramatic increase in the incidence of viral infection as the pH was lowered in laboratory cultures of blue-green algae. Alternatively, blue-green to green shifts observed may involve the differential effects of carbon limitation for these two divisions. King (1970) suggested that, at a given level of alkalinity, carbon limitation should increase with pH and that blue-greens were better adapted to low alkalinity and high pH while greens were better suited to conditions of low pH and high alkalinity. Long (1979) confirmed King's hypothesis in a series of laboratory growth experiments. He found that the half saturation constant for carbon uptake, Ks, increased with pH for a large number of species, and that, in general, blue-greens had lower Ks values than greens at a given pH. He also demonstrated the superior uptake characteristics of green algae at low pH and high alkalinity levels and predicted a shift in dominance from blue-green to green algae as the alkalinity increases and pH decreases. Figure 46 presents a summary of the conclusions of Long and King as they relate to the results obtained in experiments 1, 2, and 3. The dotted line was calculated by Long from his laboratory results. Blue-greens should outcompete greens above the line and greens should dominate below it. The analogous "transition zone" suggested by King (1970) from his observations on sewage lagoons is also shown. Long felt that the difference between his dividing line and King's was due to the higher levels of mixing which he used in his laboratory experiments compared to the stagnant conditions in the lagoons. King (1970) showed that the effects of carbon limitation could be greatly reduced by increasing the mixing rate in laboratory cultures. The

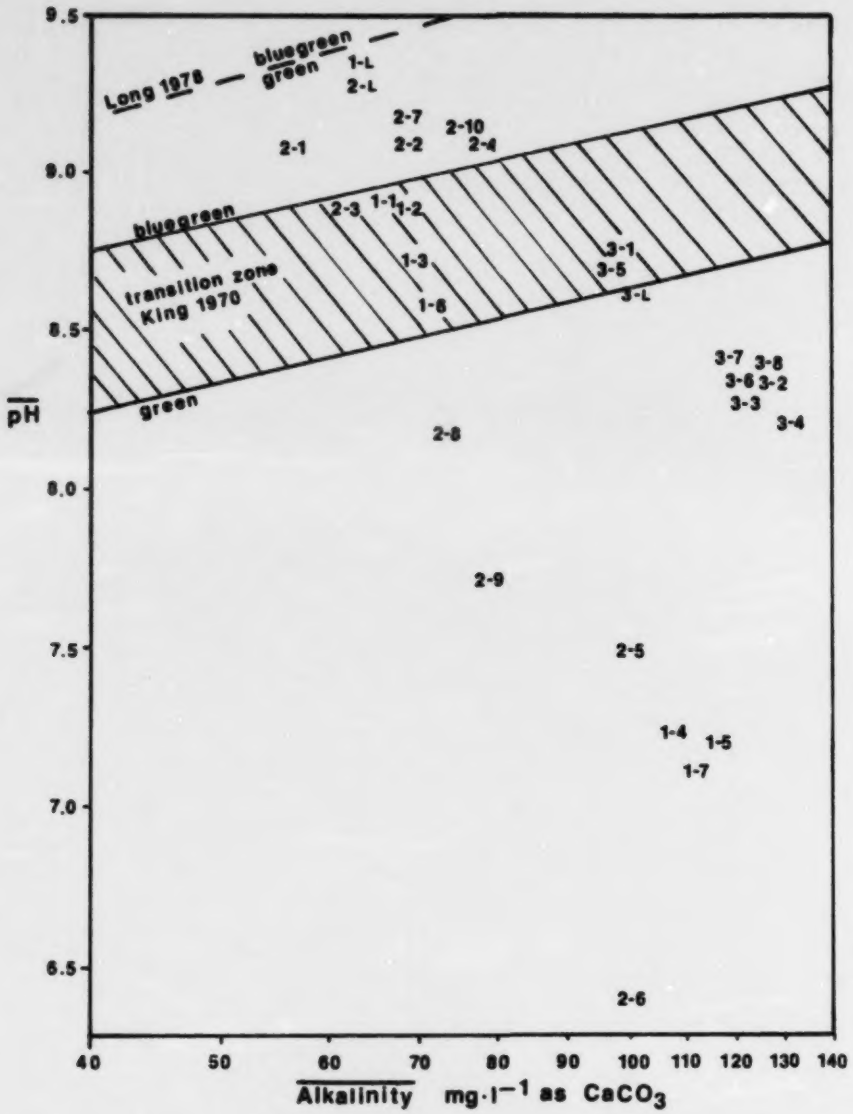

Fig. 46. Expected effect of pH and alkalinity on the competitive relationship between greens and blue-greens. (Shaded region = transition zone of King (1970), dotted line = transition boundary of Long 1978). Values of average pH and alkalinity for experiments 1, 2 and 3 are indicated by 1-J, where 1 = experiment number and J = enclosure number (L = lake).

average levels of alkalinity and pH observed in enclosures in experiments 1, 2 and 3 are also indicated on the Figures. In general, deep mixing resulted in an increase in alkalinity and decrease in pH. A wide range of alkalinity and pH levels was obtained in Little Lake Johanna (expt. 1 and 2) while a smaller range was observed in Twin Lake (expt. 3). Changes in species composition observed in these experiments were not entirely consistent with the predictions of Long and King. The values of pH and alkalinity in all of the enclosures for all three experiments were within the range which Long predicted should lead to dominance by green algae. However, while shifts in either the relative abundance or competitive advantage of greens were observed in several of these enclosures there were also many instances, most notably in bags 2 and 7 in experiment 2 and all of the bags in experiment 3, where blue-green algae dominated. The results are somewhat more consistent with the predictions of King in that the bags in experiment 2 which were dominated by blue-greens occurred above his transition zone. However, the alkalinity and pH levels for Little Lake Johanna in experiments 1 and 2 were also in this region (1-L, 2-L) and these communities were dominated by green algae. In addition, most of the enclosures in Twin Lake, which were dominated by blue-greens still fell within the "green" region. While the lack of agreement between experimental results and the predictions of Long and King is difficult to explain, there was one trend in the experimental data which suggests that carbon limitation may still be involved in the pH shift mechanism during artificial circulation. This was the shift in competitive advantage from blue-greens to greens which occurred in the deep mixed enclosures in experiment 1 (Fig. 21). This shift was accompanied by a large drop in pH and increase in alkalinity. While green algae never fully gained the advantage over the blue-greens at the faster mixing rate, this result suggests that a shift in species composition might occur if the pH fell much further.

An increase in diatoms was also observed in several of the rapidly mixed enclosures. Similar increases in diatom species have also been reported in whole lake destratification experiments (Bernhardt 1967, Haynes 1975, Knoppert _et al_. 1970, Lackey 1973, Weiss and Breedlove 1973). As with the green algae, the advantage of diatoms at the faster mixing rates may have been related in part to a reduction in carbon limitation resulting from lower pH and higher CO2 concentrations. However, the results obtained in experiment 1 indicate that another mechanism may have also been involved. The growth rates of all species increased dramatically in the "shallow mixed" enclosures without a major change in the levels of pH, CO2, alkalinity or TP/Chl _a_ (Fig. 21) suggesting that neither carbon nor phosphorus limitation were involved. These increased growth rates were apparently related to the direct effects of turbulent mixing. However, the mechanism involved is not clear. Increased turbulence may have reduced nutrient limitation indirectly by breaking down the zones of nutrient depletion which can surround algal cells under quiescent conditions (King 1970, Munk and Riley 1952). Alternatively, the sinking velocities of algal cells may have been reduced as the mixing rate increased resulting in lower sinking losses. This latter mechanism would have had the most significant effect for the heavier diatom species. The fact that diatoms showed the greatest increase in growth rate in the shallow mixed bags suggest that this might be the principal mechanism involved. Knoechel and Kalff (1975) found that seasonal changes in turbulence had a significant differential effect on the sinking losses of a blue-green and a diatom species in Lac Hertel, Quebec, resulting in a dramatic shift in their relative dominance. It is also possible that, in our system, the increase in diatoms at the faster mixing rates was an "enclosure effect" caused by the dislocation and suspension of periphytic species growing on the side walls. The enclosure walls often became covered by a dense layer of periphyton, predominantly diatoms, by the end of an experiment. This increase in periphyton often coincided with the shifts to diatom species observed in the plankton. The fact that many of the diatom species which occurred in the plankton were also periphytic forms (e.g. *Synedra* spp. *Nitszchia* spp.) suggests that these shifts may have been artificial.

Oscillatoria rubescens was the dominant alga in Twin Lake during all of the Twin Lake experiments. This alga commonly resided in the metalimnion and was therefore only observed in the open bottom enclosures, and in the lake. It generally responded favorably to mixing and its population usually increased in proportion to the total phosphorus concentration (Figs. 26, 39, 45). Smith *et al*. (1975) also found that metalimnetic populations of *Oscillatoria rubescens* increased following destratification in eutrophic Mirror Lake, Wisconsin. However, Bernhardt (1967) observed a dramatic decline in this species following destratification in Wahnbach Reservoir and a similar reduction was observed in a metalimnetic population of *Oscillatoria tenuis* during artificial circulation (Weiss and Breedlove 1973). The autoradiographic results obtained in experiment 3 indicate that the average photosynthetic rate of the *Oscillatoria* population increased dramatically after destratification (Fig. 27). Baker *et al.* (1969) also found an increase in photosynthesis when they took samples from a metalimnetic population of *Oscillatoria agardhii* and incubated them near the surface. In a similar experiment Watanabe (1979) observed higher growth rates for *Oscillatoria mougeotia* when samples taken from the metalimnion were incubated near the surface. He felt that these higher growth rates were due to either the higher light levels or higher temperatures found near the surface, or both. The increase in photosynthesis observed for *Oscillatoria rubescens* in the mixed bags, then, was apparently the result of increases in both the temperature and available light which occurred as the metalimnetic population was brought to the surface. Once it was "released" from light limitation, the population grew to a level determined primarily by the concentration of total phosphorus in the mixed layer (Fig. 26). The response of *Oscillatoria rubescens* was not consistent with the prediction of equation 11 that the population should decrease as the mixed depth is increased due to increased light limitation. This contradiction occurred because one of the assumptions of the model was violated. The model assumes that the algae are uniformly distributed through the mixed layer while, in this case, they were concentrated in the metalimnion.

Mechanisms Involved in the Community Response During Artificial Circulation

The evaluation of the mathematical model presented above with data from experiment 6 indicates that this model provides the basic theoretical framework necessary to understand the community response during artificial circulation. This framework is summarized by equation 11. The parameters in this equation, most likely to change during destratification are the mixed depth, Zm, and the concentration of total phosphorus, TP, in the mixed layer. The mixed depth always increases during artificial circulation while the results of our experiments suggest that TP also usually increases. Equation 11 predicts that these two changes will have opposite effects on the maximum concentration of chlorophyll attained in the mixed layer, c*. An increase in TP will cause c* to increase while an increase in Zm causes c* to decrease. These contradictory effects of Zm and TP provide a mechanistic explanation for the ambiguous results obtained in previous whole lake destratification experiments. It is clear that c* can either increase, decrease or remain the same following destratification depending on which factor, TP or Zm, has the greater effect. This was clearly demonstrated in the simulation for Twin Lake (Fig. 40b). Since destratification of the experimental enclosures resulted in a large increase in TP and only a small increase in Zm, c* increased dramatically. As with the population density, c*, the total algal population in the mixed layer, c*Zm, is also expected to increase if TP increases following destratification. However, c*Zm, may either increase, decrease or remain the same, as Zm changes depending on both the initial and final value of Zm. This general response pattern was shown in the simulation for Twin Lake (Fig. 40b). The small increase in Zm and the large increase in TP observed in the mixed experimental enclosures both acted to increase the value of c* Zm and it is expected that a similar result would be obtained if the whole lake was destratified. An analysis similar to the one given for Twin Lake was presented by Lorenzen and Mitchell (1975) for Kezar Lake, New Hampshire. They considered the effects which nutrient and light limitation would have on algal growth during artificial circulation and although they used a different model their results were qualitatively the same. They also showed that peak biomass, c*Zm, would either increase, decrease or remain the same depending on the initial and final value of Zm. As in the analysis for Twin Lake, they predicted that peak biomass, c*Zm, would reach a maximum at a specific mixed depth which they had defined in a previous paper (Lorenzen and Mitchell 1973) as the optimal mixed depth, Z-opt. However, the value of Z-opt determined for Kezar Lake (approx. 1.5 m) was much less than the value determined for Twin Lake (approx. 15 m). This lower value of Z-opt in Kezar Lake means that a much smaller increase in Zm would be required to cause a reduction in c*Z. This difference in Z-opt, then, indicates that the sensitivity of algal communities to destratification can vary significantly between lakes. While both analyses predicted a qualitatively similar response to changes in Zm, Lorenzen and Mitchell (1975) did not consider the effects of changes in nutrient concentration in their analyses. The results of the simulation for Twin Lake indicate that these changes can have a significant effect on the community response.

The results obtained in the other experiments also indicate the importance of changes in nutrient concentrations to the community response during mixing. The concentration of total phosphorus in the mixed layer always increased when Zm was increased in the open bottom enclosures. These increases in TP usually resulted in increased concentrations of chlorophyll a in the mixed layer. Significant positive relationships were found between the concentrations of TP and Chl a in the mixed layers of enclosures in six of the eight experiments described. The regression lines obtained from these relationships are shown in Figure 47. The regression lines determined for experiments on the same lake generally have similar slopes and intercepts (experiments 3, 6 and 8 were on Twin Lake; experiments 1, 2 and 5 were on Little Lake Johanna). However, there were significant differences ($P = .05$) in both the slopes and intercepts of these relationships between lakes. A given increase in TP resulted in a much greater increase in Chl a in Twin than in Little Lake Johanna. This difference in yield was apparently related to a difference in inorganic N: inorganic P (IN/IP) ratios between lakes. The IN/IP ratio was determined initially in most experiments on an integrated sample taken through the depth of the expected mixed layer. This value is shown in brackets for the experiments in Fig. 47. The values of IN/IP for Twin Lake experiments were all above 30 while the values for Little Lake Johanna experiments were all below 7. Forsberg *et al*. (1978) surveyed a large number of Swedish lakes and found that phytoplankton growth was limited by phosphorus when IN/IP was greater than 12, nitrogen when IN/IP was less

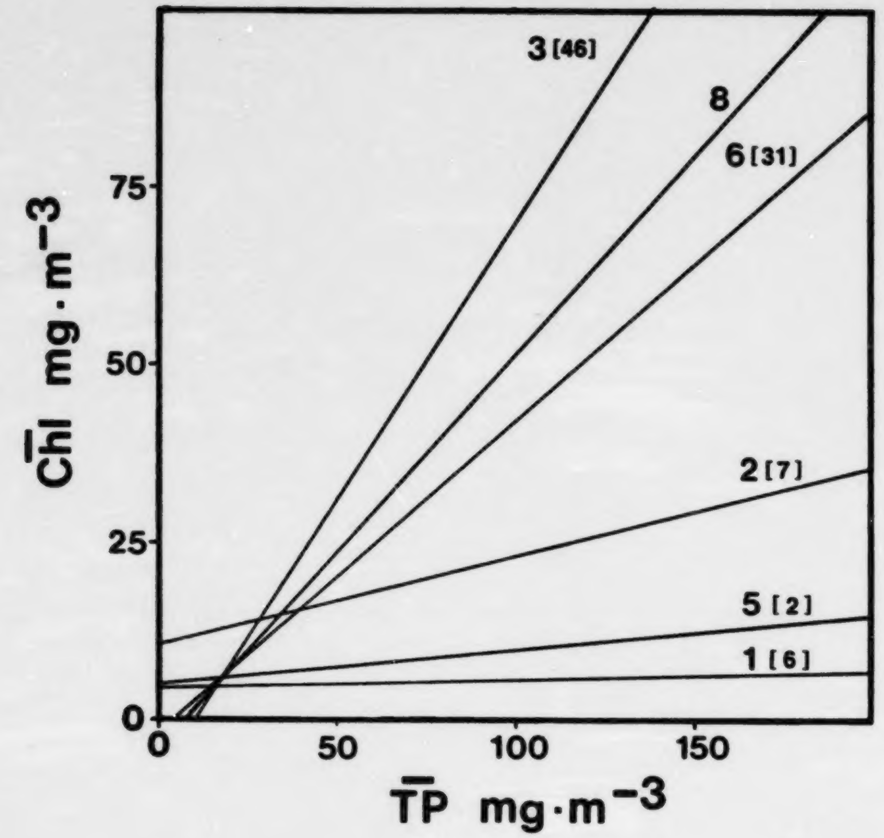

Fig. 47. Regression lines from relationships between average TP and chlorophyll a concentrations determined for experiments 1, 2, 3, 5, 6 and 8 (numbers in parenthesis indicate the initial ratio of inorganic nitrogen/inorganic phosphorus in each experiment).

than 5, and could be limited by either if IN/IP was in the range 5-12. It is clear, then, that the phytoplankton in Twin Lake were limited by phosphorus while the low yield and low IN/IP observed in Little Lake Johanna suggests that these phytoplankton were limited by nitrogen. These results indicate the importance of knowing which nutrient limits algal growth since this will be the nutrient which mainly influences the community response during mixing. The mathematical model presented above will have to be modified to describe the community change in N-limited lakes during artificial destratification. This may be done by re-defining the model parameters, TP, Kq and Pmax in terms of total nitrogen. However a test of this "total nitrogen" model will have to be made before it can be recommended for routine use.

In addition to Zm and the concentration of limiting nutrient, the loss rate D may also change during artificial circulation. In the theoretical framework an increase in D would result in a decrease in c* (equation 11). Although D was fairly constant in experiment 6, small differences in the grazing rate between enclosures did affect the final yield of chlorophyll (Fig. 41) with the lowest yield observed in the enclosure with the highest grazing rate. Oskam (1978) also found an inverse correlation between the abundance of zooplankton and the yield of chlorophyll *a* in several destratified eutrophic reservoirs. Several investigators have reported increases in the abundance of herbivorous zooplankton during whole lake circulation (Riddick 1957, Brown *et al*. 1971, McNall 1971, Shapiro and Pfannkuch 1973). These changes might also be expected to result in lower yields of phytoplankton. However, the final concentration of chlorophyll achieved will always depend on the combined effect of changes in D, Zm and the concentration of the limiting nutrient as indicated in equation 11.

The constants: Pmax, Kq, θ, ln (Io/Iz') and Ec in equation 11 should change with community composition while the constant Ew will depend on the amount of non-algal material dissolved or suspended in the water. These "constants" determine how changes in the concentration of limiting nutrient, Zm and D which occur during mixing will influence the community response. Since community composition and Ew can vary significantly both between lakes and within a given lake it will be necessary to perform a separate analysis for each lake and community considered. It will also be necessary to consider the effects which changes in species composition during artificial circulation will have on the model constants.

The low yield of algal biomass per unit of TP which was often observed at the fastest mixing rates (experiment 1, bag 7; experiment 4, bag 10; experiment 7, bags 8, 9, and 11) is difficult to explain. This apparent inhibition of growth may have been related to the low surface temperatures achieved in these bags. A surface temperature of 12.4 degrees C was produced in bag 7 of experiment 1 at a mixing rate of 1000 ml min-1. This was much lower than the value of 23 degrees C obtained at a mixing rate of 100 ml min-1 (bag 5), although the surface levels of pH and nutrients achieved in the two bags were quite similar (c.f. Figure 21). If temperature were the only factor responsible for the difference in composite growth rates in Experiment 1 then a simple temperature correction made by assuming a Q10 value of 2.18 (Goldman and Carpenter 1974) and normalizing all growth rates to a common temperature should give similar results in both enclosures. When the composite growth rates for diatoms in bags 5 and 7 are both normalized to 23 degrees C, they are, in fact, quite similar (0.54 and 0.57 day-1, respectively). However, when the same correction is applied to the composite growth rates of blue-greens and greens a similar correspondence is not obtained (for bags 5 and 7, respectively: blue-greens: 0.13 and 0.022 day-1; greens: 0.06 and 0.018 day-1). There is apparently some other factor in addition to temperature which is contributing to the lower growth rates for these latter divisions at the faster mixing rate. The fact that all of the rapidly-mixed low-yield enclosures were open at the bottom suggests that some hypolimnetic factor may be involved. Symons et al. (1969) also observed some inhibition of algal growth during whole lake circulation. They suggested that increased concentrations of hydrogen sulfide which they noted at the surface during mixing may have been responsible for the effect. The fact that hydrogen sulfide was also found at the surface of bag 7 during experiment 1 suggests that a similar mechanism may have been involved in the inhibition of blue-greens and greens in this enclosure. Clearly, further research into the effects of temperature and hypolimnetic constituents on algal growth is needed before this problem can be fully resolved.

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II. C. EFFECTS OF ENVIRONMENTAL STRESSES ON THE RELATIONSHIP BETWEEN *PLECTONEMA BORYANUM* AND CYANOPHAGE LPP-1*

INTRODUCTION

For many years a reduction of nutrient input to lakes was considered the universal remedy to combat the cultural eutrophication created during the last decades. Although meaningful, the measures were not sufficient in several cases (Bjork *et al*. 1972, Ryding and Forsberg 1976), and additional alternative approaches are in demand (Shapiro 1977, 1978).

Changing phytoplankton communities from blue-green algal dominance to green algae with the intention of improving the algal food resource for higher organisms in the ecosystem is an attractive approach which was successfully accomplished by Shapiro (1973) and Shapiro *et al*. (1975). In their experiments, performed on a small scale in the field (plastic enclosures), a rapid shift from blue-green algae to green algae followed an injection of CO2 and the accompanying drop in pH. Whether the supply of CO2 favored the green algae in competition with blue-green algae for carbon, or whether other organisms were affected and involved in the mechanism of the shift is still unclear. Artificial circulation of whole lakes has sometimes resulted in a shift from blue-green to green algae or diatoms. The successful ventures generally were accompanied by a lowering of pH (Shapiro 1977).

The recent attention focused on the microbial pathogens of blue-green algae and their interrelations has exposed an attractive field where many questions remain despite intense research efforts (Safferman and Morris 1963, Stewart and Brown 1969, Safferman 1973b, Padan and Shilo 1973, Daft *et al*. 1975, Stewart and Daft 1977). Several investigators (Safferman and Morris 1964, Jackson and Sladecek 1970, Cannon *et al*. 1974, Cannon 1975) have commented on the use of viruses as a means of controlling algal blooms. However, Goryushin (1976) warns of the ability of cyanophages to induce mutation in higher organisms. Nevertheless, the presence of cyanophages in environments common to many blue-green algae, e.g. at high pH, must be significant as a selective factor.

The present study is an attempt to clarify whether relations between viruses (cyanophages) and their algal hosts can be affected by manipulations in the environment. Is it possible to activate cyanophages and accelerate lysis of blue-green algal populations or to enhance the resistance of blue-green algae to attack from cyanophages?

The experiments presented here were performed under laboratory conditions with a well-known algal-cyanophage system, *Plectonema boryanum* and cyanophage LPP-1 (attacking strains of *Lyngbya*, *Phormidium* and *Plectonema*). The work was done in close connection with field experiments on natural blue-green algae communities, however, because the nature of the induced blue-green algal collapse in plastic enclosures suggested lysis of the algal cells (Shapiro *et al*. 1975, and pers. commun.).

The rate of LPP-1 cyanophage replication and lysis of *Plectonema* was studied in relation to:

a) pH alterations by CO2/air additions
b) algal host culture age and density
c) nutrient concentrations
d) presence of additional algal species

The results will be discussed in relation to observations from field experiments (Shapiro 1973, Shapiro 1977).

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*by Gunilla Lindmark and Joseph Shapiro

MATERIALS AND METHODS

Algal and Cyanophage Strains

The filamentous blue-green alga, *Plectonema boryanum* IU 581 (Indiana Univ. Culture Collection) and its cyanophage LPP-1 (kindly supplied by Dr. R.E. Cannon at the University of North Carolina, Greensboro) were the main organisms selected for the experiments. Morphological and physicochemical characteristics of the LPP cyanophage and its algal hosts and the process of viral infection are reviewed by Padan and Shilo (1973). Additional algal strains used were cultures of the blue-green alga *Anabaena circinalis* (Wis 1038) and the green alga *Scenedesmus* sp. (probably *S. obliquus*, Rhee 1972).

Culture Methods

Algal stock cultures were grown in a synthetic nutrient solution (modified Gorham's medium, Table 17), and algal cells were routinely transferred into fresh nutrient solution at intervals of 10 days. Cells from 12-day old stock cultures (density ca. 20 million cells/ml) were used as inoculum in the experiments. Both stock and experimental algal cultures were incubated at 24± 2 degrees C and were constantly illuminated by cool-white fluorescent lamps with an intensity of 1500 lux. The stock cultures were hand shaken once daily while cultures in the experimental flasks were mixed for 7 minutes once an hour by teflon-coated magnetic stirring bars.

Cyanophage suspensions were obtained by lysis of a host algal culture and filtered (Millipore 0.22 um). The suspensions were stored refrigerated and in the dark to prevent losses in viability (Padan and Shilo 1973). The titer of the cyanophage suspension was assayed prior to every experimental series. The requirement of the LPP-1 cyanophage for 10 mM MgCl2 in the medium was fulfilled in all experimental cultures.

The experiments were run as batch algal cultures with 500 ml of nutrient solution in 1-liter Erlenmeyer flasks. The flasks with nutrient solution were autoclaved with two Pasteur pipets inserted through the cotton plug and with tygon tubing attached to one of the pipets for sampling (cf. Fig. 48). Sampling was performed by inserting sterilized Pasteur pipets into the tubing and removing culture suspension by suction immediately after mixing. No attempts were made to keep the cultures completely free from bacteria. Depending on the design of the experiment, the cultures were incubated for 8-25 days. Due to limited experimental culture volumes and the time factor in viral replication, it was necessary to set priorities in the analytical program to give maximum information in a limited number of experiments.

Description of a Culture Unit

A schematic diagram of one culture unit is shown in Figure 48. In each experimental series six or seven culture flasks with separate pH meters and valves for CO2/air (5:95, v/v) were connected in parallel to a timer and the CO2 air tank. The pH meters were operating constantly during the experiment and functioned to hold pH levels below preset values. The timer activated stirring of the culture solutions and opened the valves for CO2/air bubbling seven minutes per hour. The valves were secondarily controlled by pH in the cultures, and gas was provided if pH exceeded the value set on the pH meter for a particular culture. As soon as pH levels were corrected the valves were closed. The tubing for gas supply was transparent tygon and glass when possible. Due to a pressure decrease from loss of gas by diffusion through the walls of the tubing when no gas was required for a longer time (ca. 5-6 hrs), culture solution was sucked up into the inlet pipet and occasionally

Table 17: Composition and concentration of nutrient solution*

Compound	mg/l	mM
KCl	34	0.46
$Na_2SiO_3 \cdot 9H_2O$	58	0.20
$Na_2CO_3 \cdot 10H_2O$	54	0.19
$MgSO_4$	75	0.62
$CaCl_2$	36	0.48
$NaNO_3$ 1)	496	5.80
KH_2PO_4 1)	4.7	0.032
$FeCl_3 \cdot 6H_2O$	2.8	0.01
EDTA	3.7	

Trace solution (Hoagland's A-Z)

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* modified from Hughes, Gorham and Zehnder (1958)

1) concentrations modified in some experiments

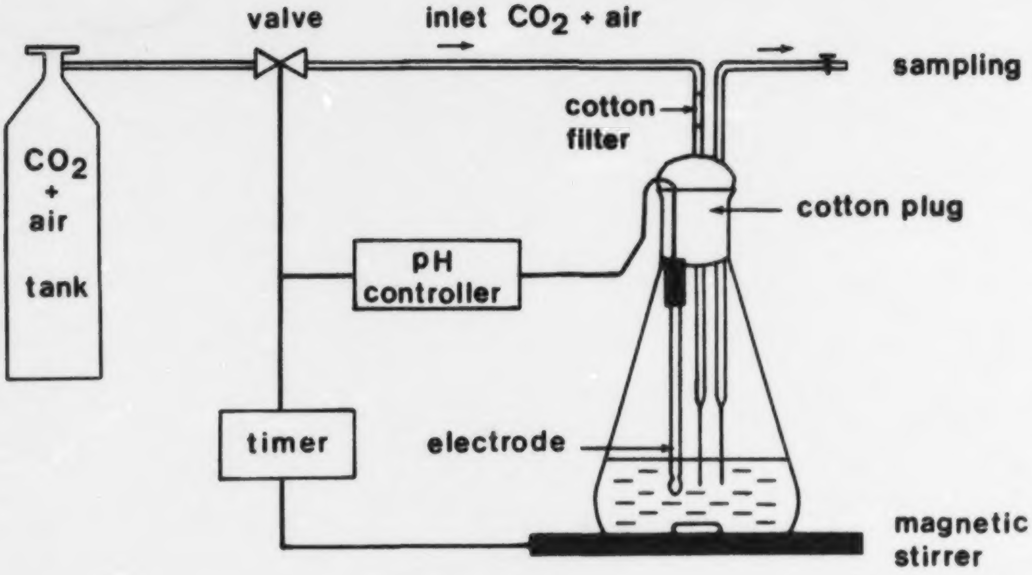
valve
inlet CO_2 + air
sampling
cotton filter
cotton plug
CO_2 + air tank
pH controller
timer
electrode
magnetic stirrer

Fig. 48. Schematic diagram of one experimental unit.

into the tubing. Various kinds of tubing were tested but none worked satisfactorily. Therefore, either the gas supply mechanism was interrupted during the night or the outlets (pipet tips) were raised above the surface of the culture suspension. In the latter case the gas supplied was not always enough for complete correction of pH increases in the cultures.

Analytical Methods

Physical and chemical analyses

pH was recorded every second to third hour during daytime. PO4-P was analyzed as soluble reactive phosphorus according to Murphy and Riley (1962) and by the stannous chloride method. NH4-N was determined according to Chaney and Marbach (1962), and NO3-N was analyzed as NO2-N (Bendschneider and Robinson 1952) after reduction by a copperized Cd column. For chlorophyll *a*, 5-25 ml of algal suspension was filtered under vacuum on a 2.5 cm glass fiber filter (Whatman GF/C) and washed with a small volume of distilled water. The filter was ground in 90% acetone, and chlorophyll was extracted for 12 hrs in the dark at 4-6 C. Estimation of chlorophyll *a* followed the method of Lorenzen (1967).

Cyanophage assays

For plaque assays, 0.5 ml portions of diluted viral suspension were mixed with 2.0 ml of a dense algal host culture and 2.5 ml of melted nutrient agar (nutrient solution in 1% agar) and plated on Petri dishes over a base of 15 ml solidified nutrient agar (nutrient solution in 1.5% agar) according to the double-agar layer technique (Safferman 1973a). The plates were inverted and incubated under continuous illumination (1500 lux) at 22± 1 degree C, and the number of cyanophage particles was estimated by counting clear plaques on the algal "lawns" after 4-5 days incubation. Appropriate cyanophage titer for assay was obtained through tenfold dilutions in a saline-magnesium solution (0.2 g MgCl2. 6H2O and 5.85 g NaCl per liter distilled water). Each plaque developed on the plate was considered to originate from one cyanophage, and the titer of the assayed suspension (plaque-forming units (PFU)/ml) was evaluated using the proper dilution factor.

EXPERIMENTAL DESIGN AND RESULTS

The experiments were performed in four successive series with six or seven culture flasks in each series. Results from one series aided in determining the next. Since the addition of CO2/air to reduce pH did not show any differences in the nature or rate of lysis of *Plectonema* cultures when compared to the use of HCl in a preliminary study, addition of CO2/air was selected for all experiments to avoid changes in alkalinity (Stumm and Morgan 1970, Brewer and Goldman 1976).

Series 1

This experiment was done to determine whether pH has an effect on the alga-cyanophage relationship. *Plectonema* was grown in the nutrient solution (at 1 mg PO4-P/l) for 11 days with no pH adjustment. The cyanophage was then inoculated, and pH adjustment with CO2/air was begun (Fig. 49). Controls received no CO2/air.

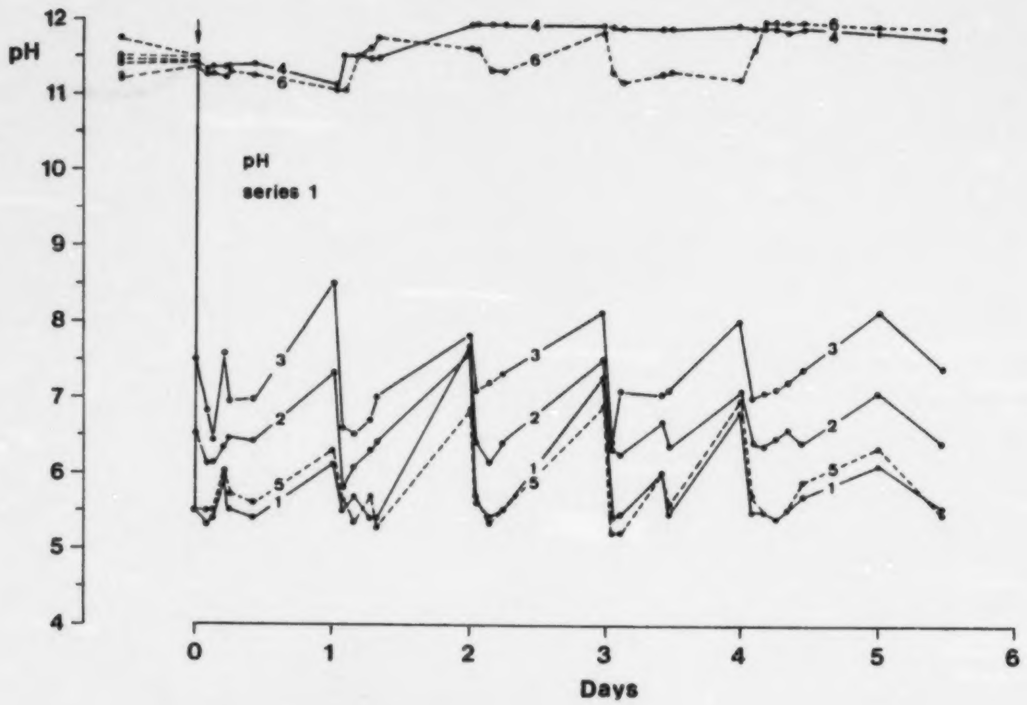

Fig. 49. pH in cultures of Plectonema infected with cyanophage LPP-1 (——, 1-4) and cyanophage-free (---, 5 and 6). Arrow (day 0) indicates inoculation with cyanophage and start of CO_2/air addition (1-3 and 5) to 11-day old cultures.

Culture no.	pH adjusted with CO2/air to:	LPP-1 added (PFU/ml)
1	5.5	100,000
2	6.5	100,000
3	7.5	100,000
4	control	100,000
5	5.5	0
6	control	0

When pH was lowered from 11.5 to about 7.5 (Fig. 49) in cultures of Plectonema simultaneously inoculated with cyanophage, replication of the cyanophage was strongly enhanced (Fig. 50). In the culture where pH was lowered to 5.5, replication was delayed. After three days of incubation, however, the cyanophage concentration was similar to that in the other cultures with pH adjustment. In the culture where pH was not adjusted (Fig. 49, culture 4) on the other hand, replication of the cyanophage declined early and ceased at a comparably lower density (Fig. 50).

The response of Plectonema to the presence of the cyanophage was obvious after only 24 hours (Fig. 51) in cultures where CO2/air was supplied and the pH lowered, while algae in the control culture (without CO2/air addition) maintained a healthy population despite an increase in cyanophage particles of several orders of magnitude (Fig. 51, cf. Fig. 50).

Plectonema cultures free from cyanophage showed the same healthy growth irrespective of the change in pH (Fig. 51). The only difference noted between the two cyanophage-free cultures was that the algal filaments became short (5-30 cells) and straight in the culture with adjusted pH, while filaments in the control culture were long (more than 30 cells) and twisted into bundles. According to Padan and Shilo (1973), however, the length of the filaments has no effect on the adsorption rate of cyanophage to Plectonema or the reproductive cycle of the cyanophage.

Two days after the viral infection of Plectonema, only one- and two-cell trichomes were left in the cultures with adjusted pH, but long (more than 20 cells) and bright green trichomes were present in the control cultures. The only symptom of viral infection in the control was that the cells swelled somewhat and were thus abnormally distinct in the filaments. After four days there were no detectable Plectonema cells in cultures with adjusted pH and cyanophage. However, 17 days after the infection (adjustment of pH stopped on day 6) some long, fine filaments were observed in the lysed cultures indicating regrowth of a "new" Plectonema population. pH had by then increased to about 9. This behavior can be compared with the repeated oscillations in Plectonema density and cyanophage LPP-1 concentration observed in continuous cultures (Cowlishaw and Mrsa 1975, Cannon et al. 1976).

When the addition of CO2/air was interrupted during the night, pH increased to the same levels in cultures with cyanophage as in cyanophage-free cultures (Fig. 49). In control cultures (no CO2/air addition) the presence of the cyanophage did not affect pH conditions during the experiment (Fig. 49, cultures 4 and 6). According to Padan et al. (1970), cyanophage infection begins to inhibit photoassimilation in Plectonema cultures early in the infective process. Wu and Shugarman (1967), however, stated that the rate of photosynthetic oxygen evolution does not change until the infected cells rupture.

Thus, the present results show clearly that although the alga can live at low pH, and the alga and cyanophage can coexist at high pH, lysis occurs when the pH is lowered in an infected culture.

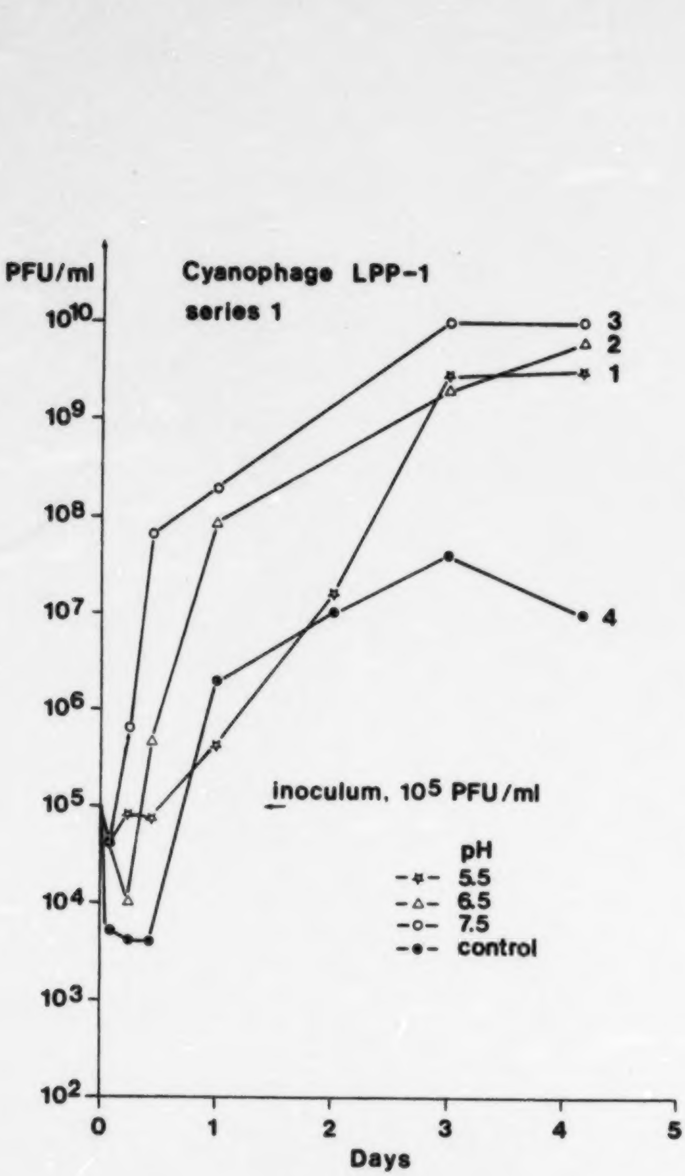

Fig. 50. Replication of cyanophage LPP-1 after inoculation on day 0 in 11-day old cultures of Plectonema. Control (●) pH adjusted (☆, △, ○). Culture numbers (1-4) indicated.

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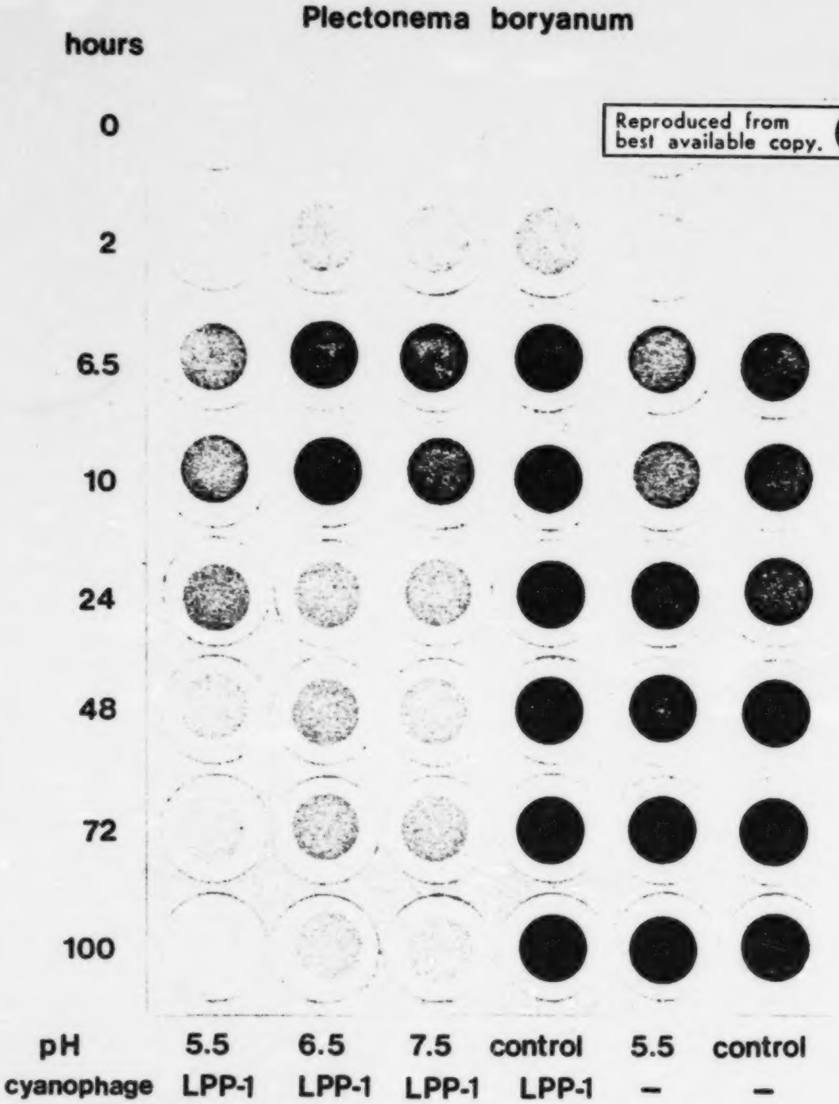

Plectonema boryanum
hours
0
2
6.5
10
24
48
72
100
pH
5.5
6.5
7.5
control
5.5
control
cyanophage
LPP-1
LPP-1
LPP-1
LPP-1
—
—

Fig. 51. Appearance of Plectonema cultures (on 0.22 μm Millipore membrane filters) growing under controlled and drifting pH conditions with and without inoculated cyanophages (LPP). Hour 0 indicates cyanophage inoculation and addition of CO_2/air to varying pH in the 11 days old cultures (Series 1).

Phosphate concentrations were below 10 ug PO4-P/l in all systems throughout the experiment (i.e. for 6 days after cyanophage inoculation).

Series 2

The purpose of this experiment was to repeat parts of series 1 using a more dilute algal culture and to determine whether continuous pH adjustment is necessary to cause lysis of the host alga. In addition, a more dilute medium was used. Fewer cyanophage particles were added to give a number of phage particles per algal cell similar to that in series 1.

Plectonema cells (35 ml of a 12-day old stock culture) were inoculated into a half-strength (0.5 mg PO4-P/l) nutrient solution (Table 17). This was stirred 3 h for pH stabilization before cyanophage LPP-1 was inoculated and CO2/air addition begun (Fig. 52). In two cultures CO2/air injections were performed only one and three times, respectively.

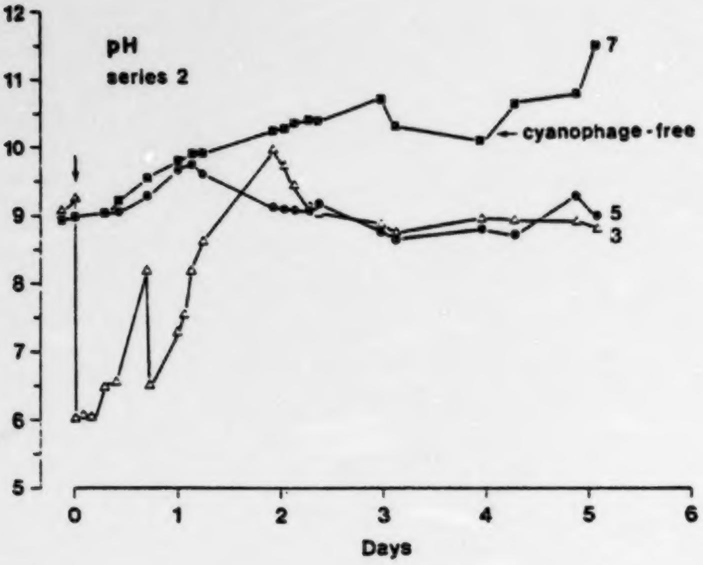
Culture no.	pH adjusted with CO2/air to:	LPP-1 added (PFU/ml)
1	6	2000
2	7	2000
3	6 (3 times)	2000
4	6 (once)	2000
5	control	2000
6	6	0
7	control	0

In this series the fastest replication of cyanophage occurred in the culture where no CO2/air was added (Fig. 53, control culture). Because of the dilution of the algal inoculum, however, pH values in the cultures were more than 2 pH units lower than in the first series at the time the cyanophage was inoculated (Fig. 52, cf. Fig. 49). Furthermore, pH was controlled by cyanophage activity after one day of incubation, since photosynthetic activity was affected (Fig. 52). In the cyanophage-free control culture pH increased as a function of algal growth (Fig. 52, cf. Fig. 54).

In the cultures with pH adjustment cyanophage replication was delayed, but it later progressed at a similar rate in all cultures (Fig. 53). As in the earlier experiment (series 1), the "most acid" culture (pH ca 6) contained a lower concentration of cyanophage during the first two days of viral infection than did the cultures where pH was kept around 7 (Fig. 53).

Chlorophyll a development in the cultures was inversely related to cyanophage replication (Figs. 53 and 54). The rapid lysis of Plectonema cells in the control culture (no CO2/air addition) directly reduced the uptake rate of PO4-P (Fig. 55, culture 5). Cultures with one or three injections of CO2/air were able to absorb more PO4-P before being affected by cyanophage activity (Fig. 55, cultures 3 and 4). In the cultures with pH adjustment and cyanophage, only small amounts of PO4-P were left in solution, and only a slight increase in PO4 could be seen on about the seventh day of the infection period (Fig. 55). The concentration of phosphate was initially 500 ug P/l; however, three hours after addition of algal cells, i.e. at the time for cyanophage inoculation, only 200 ug PO4-P/l remained in solution. The cyanophage-free cultures depleted PO4-P from the nutrient solution within three days (Fig. 55).

Two days following cyanophage inoculation NH4-N appeared in solution in some cultures (Fig. 55). Since the culture which lysed first showed the



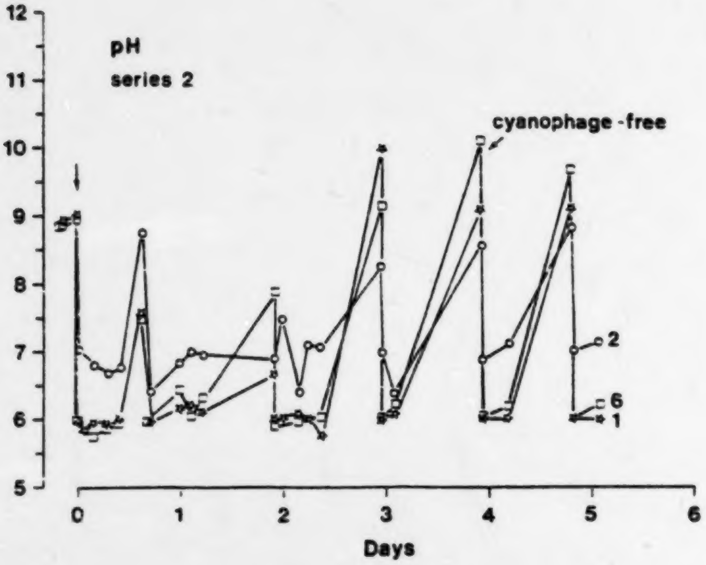
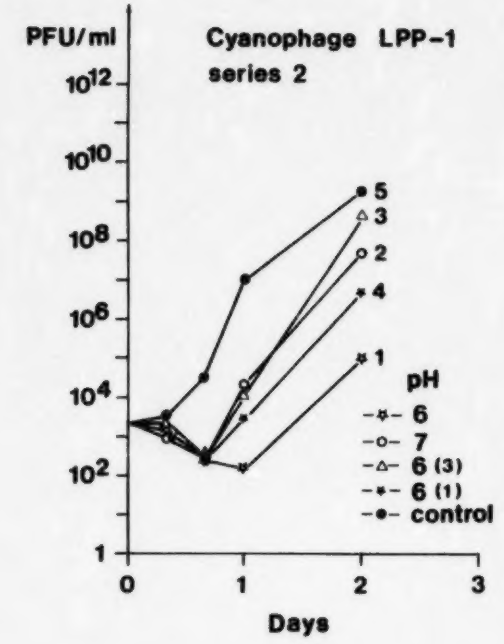


Fig. 52. pH in cultures of Plectonema infected with cyanophage LPP-1 (1-3 and 5) and cyanophage-free (6 and 7). Arrow (day 0) indicates inoculation with cyanophage and start of CO_2/air addition (1-3 and 6). Culture numbers are indicated.



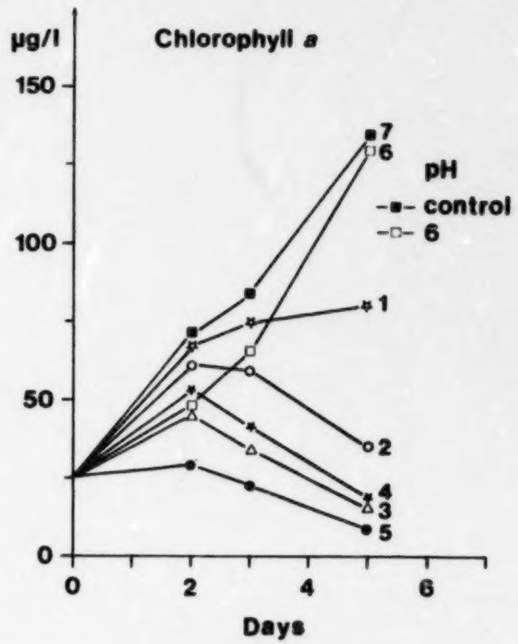


Fig. 53. (left). Replication of cyanophage LPP-1 in Plectonema cultures after inoculation on day 0. Control (●), pH adjusted (to value shown) (✡, ○, △, ★). Culture numbers (1-5) shown. (1) and (3) indicate 1 and 3 injections of CO_2/air.

Fig. 54. (right). Chlorophyll a in cultures of Plectonema infected with cyanophage LPP-1 (✡, ○ △, ★, ●) and cyanophage-free (□, ■) pH conditions shown here and in Fig. 53. Culture numbers (1-7) indicated.

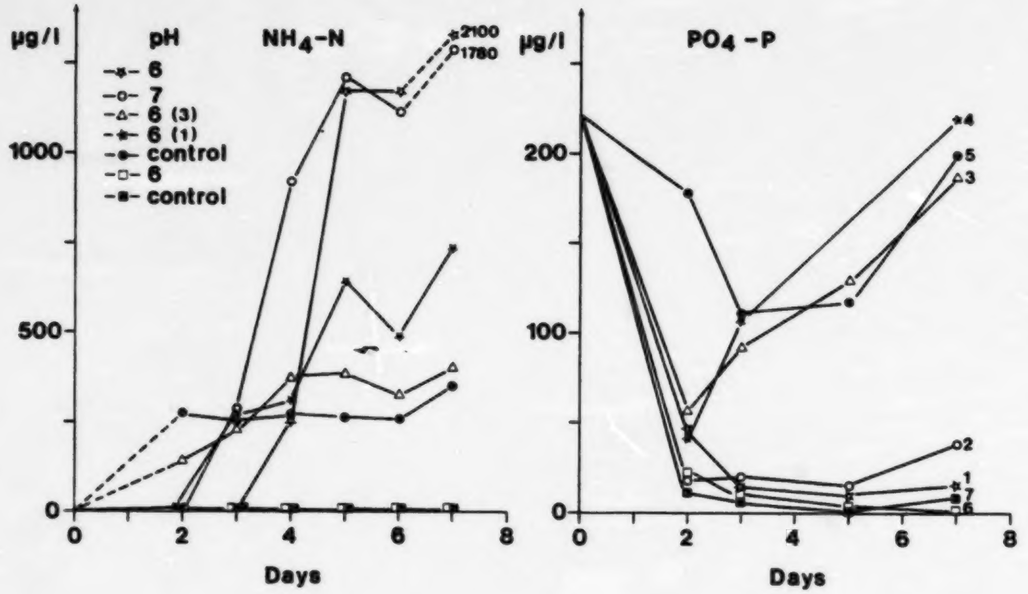


Fig. 55. Ammonia (left) and phosphate (right) in cultures of Plectonema infected with cyanophage LPP-1(✡ , ○, △, ✱, ●)and cyanophage-free (□, ■)grown under varying pH conditions (series 2). Day 0 indicates inoculation with cyanophage and start of pH adjustment. Culture numbers (1-7) shown. (1) and (3) indicate 1 and 3 injections of CO_2/air.

earliest indication of NH4-N, this was probably connected to cyanophage activity. No NH4-N was found in uninfected cultures (Fig. 55). Cultures with frequent input of CO2/air showed a rapid increase to high concentrations of NH4-N, most probably due to algal lysis. The nitrogen source in the nutrient solution was NO3, and the N:P ratio (by weight) was 80:1.

The replication rate of cyanophage in the cultures was not affected by making the CO2/air injections once, three times or frequently. With respect to PO4-P and NH4-N release following lysis, on the other hand, the frequency of CO2/air addition was critical (cf. Fig. 55).

Series 3

Because of the differences in results between series 1 and 2, i.e. the virus replicated more at ambient pH in series 2, the algal cultures used in this series were again older and more concentrated than in series 2.

Plectonema (10 ml inoculum) was allowed to grow in a half-strength nutrient solution modified to 5 mg NO3-N/l and 0.35 mg PO4-P/l for 4.5 days before cyanophage was inoculated and CO2/air addition was started (Fig. 56). In this series 1 ml of 12-day old stock cultures of *Scenedesmus* or *Anabaena* was added simultaneously with the cyanophage inoculation.

culture no.	pH adjusted with CO2/air to:	LPP-1 added (PFU/ml)	Additional alga
1	6	10,000	-
2	6	10,000	*Scenedesmus*
3	6	10,000	*Anabaena*
4	6	0	-
5	control	10,000	-
6	control	10,000	*Scenedesmus*
7	control	10,000	*Anabaena*

The replication of cyanophage was much slower in these cultures than when the host algae had approached a stationary phase of growth (Fig. 57, cf. Fig. 50) or when a surplus of inorganic nutrients was present in the culture (cf. Fig. 53). As in series 1 where no external nutrients were present, a faster replication of cyanophage LPP-1 occurred in the cultures with pH adjustment. In addition, pH levels in the cultures were similar to those in series 1 at the time of cyanophage inoculation (Fig. 56, cf. Fig. 49).

Anabaena or *Scenedesmus* added to the *Plectonema* culture simultaneously with cyanophage in this series did not significantly affect the cyanophage replication pattern (Fig. 57). Nor did chlorophyll *a* concentrations in the cultures show any differences during the first three days that could be related to algal species composition (Fig. 58). The main differences were due to the CO2/air addition and the related variation in cyanophage activity.

Neither PO4-P nor NH4-N could be detected during the first three days of infection. On the eighth day, however, 210 ug/l and 190 ug/l of NH4-N were found in the cultures with pH adjustment containing *Plectonema* and *Plectonema* + *Anabaena*, respectively. On the other hand, no NH4-N was found in the culture with pH adjustment containing *Plectonema* and *Scenedesmus* or in any of the cultures where pH was high. NO3-N and NO2-N were below 25 ug/l and 10 ugN/l, respectively, in all cultures. Initial input of NO3-N and PO4-P was much lower in this series, i.e. 5 mg N/l and 0.35 mg P/l.

After two days of incubation with cyanophage, *Plectonema* consisted of short (3-10 cells) filaments in the cultures with pH adjustment; *Scenedesmus* looked healthy, while only a small number of *Anabaena* cells remained viable. In the control cultures (no CO2/air addition) the *Plectonema* filaments after

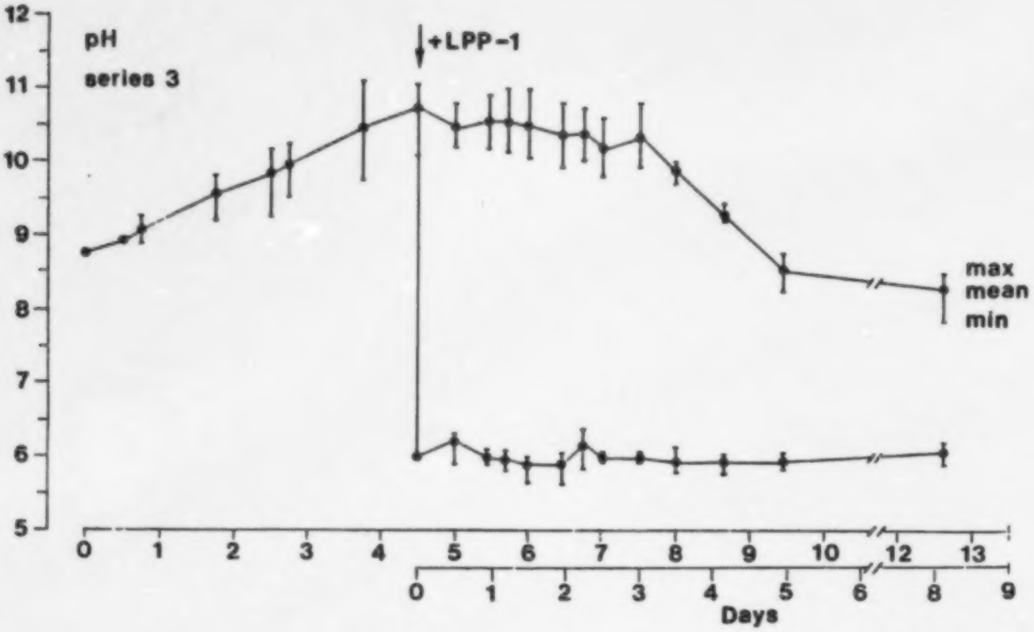


Fig. 56. pH in cultures of *Plectonema* before and after inoculation with cyanophage LPP-1 and *Scenedesmus* or *Anabaena*. Day 0 (upper scale) indicates inoculation of fresh nutrient solution with *Plectonema* cells and day 0 (lower scale) indicates start of CO_2/air addition (4 cultures) and inoculation with cyanophage (6 cultures). Dots represent mean values and vertical bars the range.

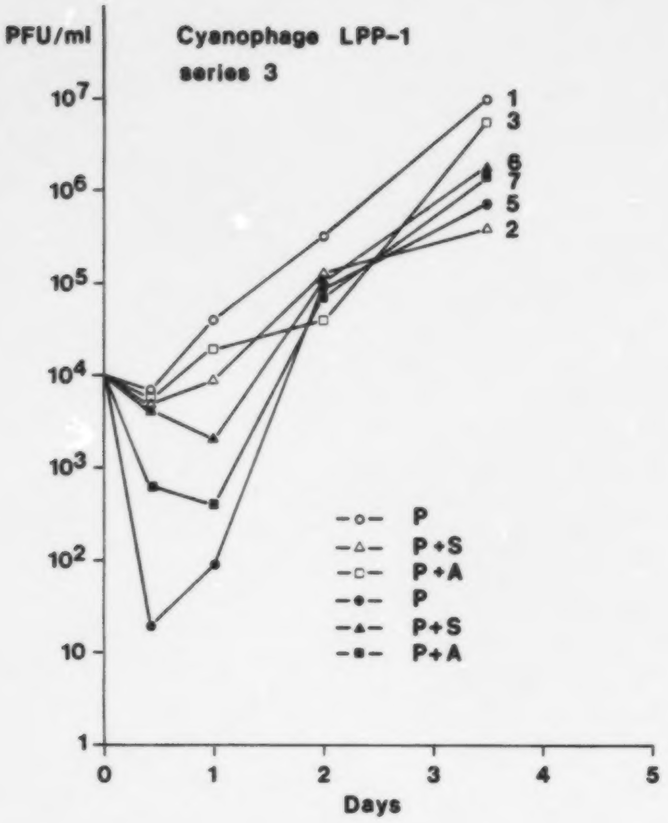


Fig. 57. Replication of cyanophage LPP-1 in algal cultures with pH adjustment (open symbols, pH ca. 6) and in controls (closed symbols). Plectonema + Scenedesmus (P+S) and Plectonema + Anabaena (P + A). LPP-1 inoculumn was 10^4 PFU/ml. Culture numbers (1-7) indicated.

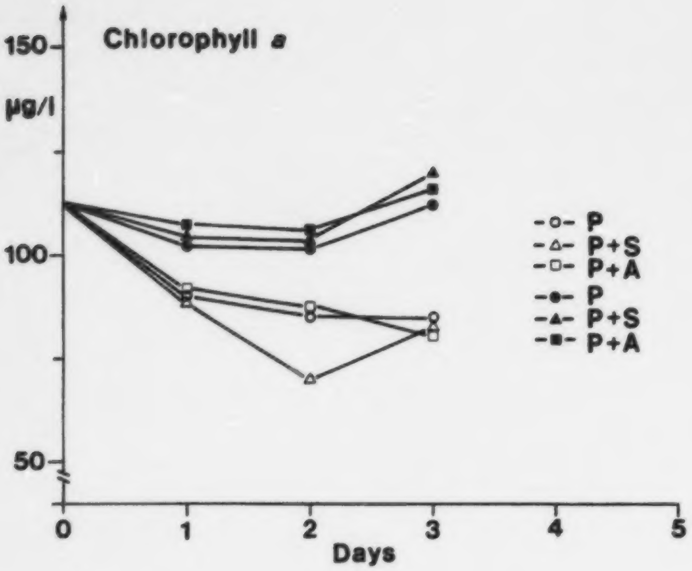


Fig. 58. Chlorophyll _a_ in cultures of _Plectonema_ (P), _Plectonema_ + _Scenedesmus_ (P+S) and _Plectonema_ + _Anabaena_ (P+A) infected with cyanophage LPP-1 and grown with pH adjustment (open symbols) or as controls (closed symbols). _Scenedesmus_ and _Anabaena_ were inoculated simultaneously with the cyanophage (day 0) in 4.5-day old _Plectonema_ cultures (series 3).

two days were long (more than 30 cells) and twisted into bundles when no additional algal species were present, and long, but not twisted, when Scenedesmus or Anabaena was present. After four days of incubation, when Plectonema in the control cultures consisted of 1-3 cell filaments and lysis was in progress, Anabaena in particular looked very healthy. Scenedesmus was more abundant in the culture with pH adjustment than in the control culture. pH in the control cultures decreased after 3 days of incubation (Fig. 56). Small amounts of bacteria were found in the lysed cultures after eight days of incubation.

Series 4

Since the age of the algal host culture seemed to affect the rate of cyanophage replication, identical nutrient solutions inoculated with Plectonema cells were allowed to reach different growth phases (considered as host age), and different densities and pH (Fig. 59) before cyanophage LPP-1 was inoculated.

Plectonema (10 ml inoculum) was allowed to grow in a half-strength nutrient solution modified to 3 mg NO_3-N/l and 0.3 mg PO_4-P/l for 1.5, 6 and 11 days before cyanophage inoculation and the start of CO_2/air addition (Fig. 59).

Culture no.	pH adjusted with CO2/air to:	LPP-1 added (PFU/ml)	Algal host culture age (days)
1	7	1000	1.5
2	control	1000	1.5
3	7	1000	6
4	7 (once)	1000	6
5	7	1000	11
6	8 (once)	1000	11

As found in earlier experiments, the cyanophage attacking algae in the exponential phase of growth showed the slowest rate of replication (Fig. 60). Again, algae in the lag phase of growth induced a rapid increase in cyanophage, indicating that variation in algal density was less important for viral replication than the physiological state of the host. The chlorophyll a concentration was 2.6 and 3.2 times higher after 6 and 11 days of initial incubation when compared with the value after 1.5 days.

The difference in cyanophage replication between one or frequent additions of CO_2/air was much smaller than that caused by algal host age (Fig. 61). Again, the infection of the "youngest" Plectonema culture resulted in a release of NH_4-N (250 ug/l on day 10 and 510 ug/l on day 15) in the culture with pH adjustment. PO_4-P was below 5 ug/l up to day 10 in all cultures, but on day 15 it ranged between 10-21 ug PO_4-P/l with no relation to host culture age or CO_2/air input.

DISCUSSION

Since Safferman and Morris (1963) isolated the first cyanophage, designated LPP since it lyses species of the blue-green algal genera Lyngbya, Phormidium and Plectonema, additional cyanophages have been discovered and investigated (cf. reviews by Padan and Shilo 1973, Stewart and Daft 1977). The main emphasis has been on cyanophage characteristics, growth cycles and interactions with host algal metabolism, mostly under standard photosynthetic conditions (Wu and Shugarman 1967, Padan, et al. 1970, Padan and Shilo 1973,

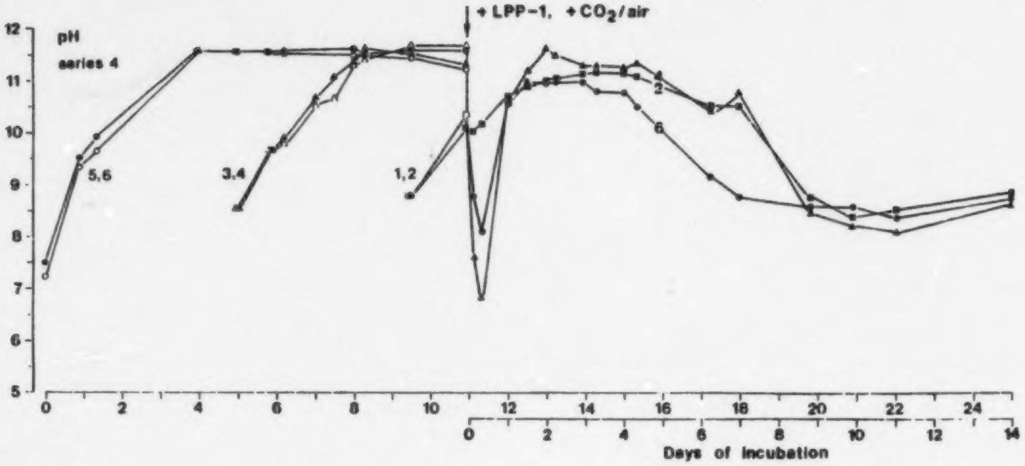


Fig. 59. pH in cultures of *Plectonema* before and after inoculation with cyanophage LPP-1 and start of CO_2/air addition (day 0, lower scale). Algae were grown for 1.5, 6 and 11 days before the cyanophage was added. Cultures with pH adjusted to ca. 7 are omitted in the diagram. Culture numbers (1-6) are indicated.

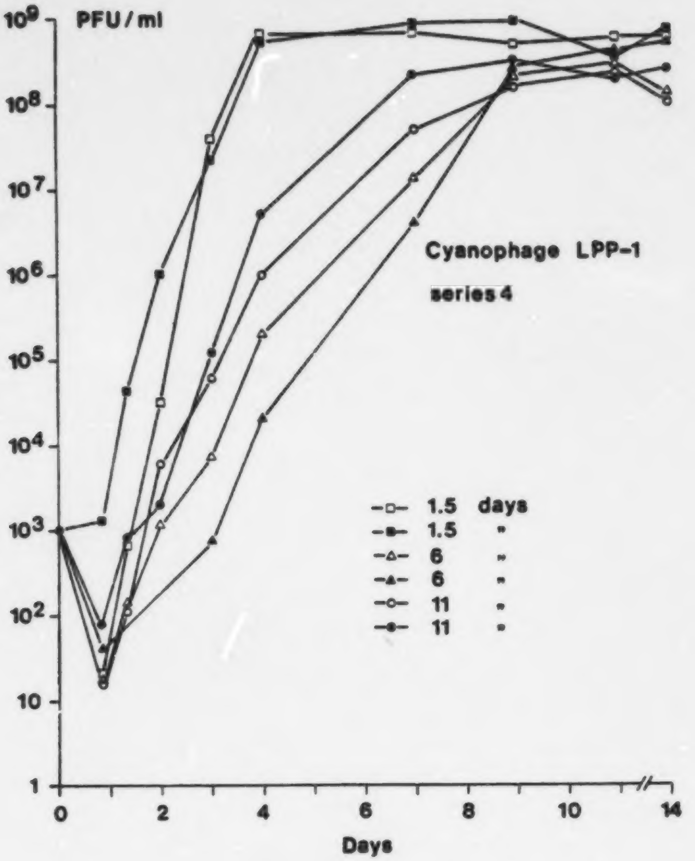


Fig. 60. Replication of cyanophage LPP-1 in cultures of *Plectonema* grown for 1.5, 6 and 11 days before inoculation with the cyanophage. Open symbols indicate cultures with frequent addition of CO_2/air (pH ca. 7), and closed symbols indicate no (■) and one (●, ▲) injection of CO_2/air.

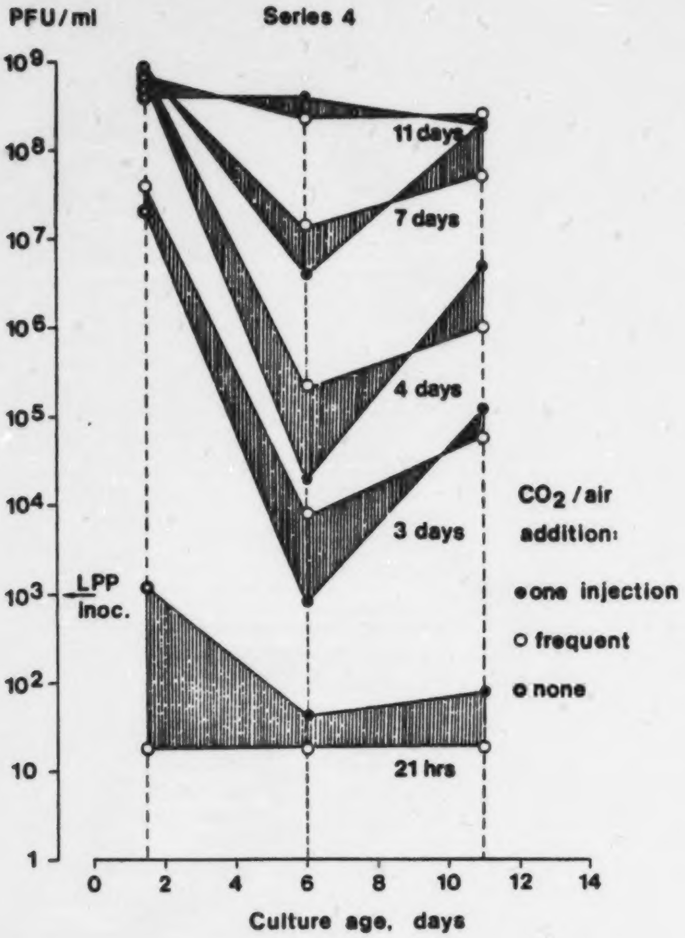


Fig. 61. Concentration of cyanophage LPP-1 in relation to Plectonema culture host age and time following cyanophage inoculation. Shaded areas indicate difference between cultures receiving no, one and frequent injections of CO_2/air.

Stewart and Daft 1977). It is clear that viral replication within the algal host is regulated largely by host metabolism, and that viral infection in turn affects the physiological state of the host alga.

Interpretation of the results from this study will be centered on how a) input of CO2/air and accompanying changes in pH, b) inorganic nutrients, and c) physiological state of the host alga (host culture age) affected the replication of the cyanophage LPP-1 in Plectonema boryanum cultures. As the conditions simulated in the laboratory were to a large extent derived from treatments used in plastic bag experiments (Shapiro 1973, 1975), the laboratory results are compared with the outcome of field experiments. Only those cyanophage - alga interactions that are more or less directly related to the areas defined above will be considered.

Dependence on pH and CO2 Input

Since growth of blue-green algae frequently occurs under high pH conditions (King 1970), and collapse can be induced by a lowering of pH (Shapiro 1973), it was interesting to find that Plectonema continued to grow at high pH levels where lysis was avoided, even though the cyanophage concentration initially increased. On the other hand, the addition of CO2/air to a culture containing the cyanophage induced a rapid lysis of the Plectonema population despite the fact that the alga could grow well at low pH in the absence of the cyanophage.

LPP cyanophage activity is reported to be stable from pH 5 to 11 (pH adjusted with HCl or NaOH in filtered viral suspensions) by Safferman and Morris (1964). The same authors reported that Plectonema develops readily at pH 7-11, whereas no, or poor growth occurs below pH 7 (cf. Padan and Shilo 1973). In my experiments the growth of Plectonema was not particularly affected when pH was lowered by addition of CO2/air to as low as 5.5 or allowed to rise to nearly 12. Instead, high (greater than 11) and very low (5.5-6) pH values seriously affected cyanophage replication, and the reduced or delayed infection of the algal cells could favour algal growth and development of chlorophyll a (cf. Fig. 51 and 54). Between pH 6 and 11 there were no significant variations in cyanophage replication patterns that could be related to pH conditions solely. According to Padan et al. (1970), replication of cyanophage LPP-1G was identical in the presence or absence of CO2.

In lysed cultures with no pH adjustment, pH stabilized between 8 and 9. Addition of Na2CO3 to lysed cultures to raise the pH accelerated a regrowth of Plectonema, while addition of nutrient solution had no effect on regrowth. Attempts to isolate Plectonema from sites where LPP cyanophages were found have proved fruitless (Cannon et al. 1974).

In field experiments (plastic bags) the induced collapse of the blue-green algal populations and the shift in species dominance occurred at pH values as high as 8.5 (Shapiro 1975). Furthermore, a reversal of the shift (to blue-greens) required re-inoculation of blue-green algae as well as increase in pH (Shapiro op. cit.).

LPP cyanophages naturally occur mostly in polluted environments, e.g. waste stabilization ponds, fish ponds during periods of fish kill, polluted rivers and rice fields (cf. Safferman and Morris 1967, Shane et al. 1972, Singh 1973, Cannon et al. 1974). Host algae have generally not been observed in samples that showed a presence of LPP virus (Safferman and Morris op. cit.). For example, along the polluted Christina River (in Delaware, U.S.A.), host algae were found only occasionally in samples where LPP cyanophages were present. A virus-resistant Plectonema laboratory culture grew well in the river water. However, the river pH was reported to be relatively stable, varying between 6.6 and 7.8, and concentrations of inorganic N and PO4-P were high (Shane _et al_. 1972). Similar observations were made in a pond close to Minneapolis (Minnesota, U.S.A.) (Lindmark unpubl.). Survival and tolerance of the LPP cyanophages apparently enable them to control their algal hosts in nature and thus eliminate the development of blooms.

Other cyanophages discovered later have been found to possess somewhat different requirements, e.g. the SM-1 cyanophage (attacking strains of _Synechococcus_) requires CO2 for replication (cf. Stewart and Daft 1977). Nevertheless, additional cyanophages need to be isolated and characterized. Just as there are specific bacteriophages for a large number of bacteria, it seems possible that there is a cyanophage for each blue-green alga.

Dependence on Inorganic Nutrients

Whether the addition of nutrients or the associated decline in pH (from 11.5 to ca. 9) favored cyanophage replication in the control culture (no addition of CO2/air) in series 2 as shown in Figure 53, is unclear. The inoculation of cyanophage in relation to algal density was about one third of that used in experiments where no nutrients were added before cyanophage inoculation. In spite of this, the amount of cyanophage (PFU) released after 24 hours was considerably higher than in series 1 (cf. Fig. 50 and 53). The addition of CO2/air in the presence of nutrients, however, resulted in a short delay in viral replication that for a short time favored the algae (cf. Figs. 53 and 54).

These results partly agree with observations from field experiments where a combined reduction in pH and addition of nutrients (N and P) accelerated the blue-green algal collapse (Shapiro 1973). In the laboratory systems the pH was lowered by the addition of nutrient solution.

From the analysis of NH4-N and PO4-P concentrations following lysis of _Plectonema_, it seems that the mode and amount of CO2/air input are important (Fig. 55). In the field the decline of blue-green algae is often accompanied by release of PO4-P (Shapiro _et al_. 1975).

When cells of _Scenedesmus_ were present during lysis of _Plectonema_, no NH4-N was detected at low pH; it was most likely utilized by _Scenedesmus_. In cultures with _Anabaena_ and without additional algal species, the released NH4-N remained in solution. It could not be determined whether low pH solely, or in combination with other factors e.g. bacteria or lysing agents (Gunnison and Alexander 1975) following lysis of _Plectonema_, was the reason that _Anabaena_ failed to survive. However, at high pH _Anabaena_ remained viable.

These findings may be useful in experiments in natural ecosystems, e.g. artificial circulation of whole lakes, with respect to nutrient requirements and competition between blue-green algae (N2 fixers in particular) and green algae.

Dependence on Age of Host Algal Culture

It is evident that _Plectonema_ in the exponential growth phase showed the strongest "physiological resistance" to lysis (cf. Fig. 61). In the 4.5 and 6-day old culture of _Plectonema_ in series 3 and 4, respectively, pH was around 11 at the time for cyanophage inoculation. The addition of CO2/air to the cultures stimulated cyanophage replication in both series. However, the resistance of the algae to lysis due to culture age was more pronounced than that due to the varying pH adjustments in series 4 (cf. Fig. 57 and 61). In quasi-continuous cultures Cowlishaw and Mrsa (1975) found that up to 0.3% of the algal cells survived the first round of lysis and suggested that this could not be due solely to mutation. Safferman and Morris (1964) reported that seven days were necessary to achieve lysis in a four-day culture of *Plectonema*.

Higher resistance to viral attack during the exponential phase of growth can be compared to the usual pattern of development of blue-green algae. In Lake Erken (Sweden) *Aphanizomenon flos-aquae* steadily increased during summer before a viral infection terminated the bloom in September (Granhall 1972). Physical and chemical environmental factors (no pH values reported) varied little during the critical period; however, the abundance of *Aphanizomenon* was extremely low the following summer.

CONCLUSIONS

Although several interactions in the cyanophage-alga system studied paralleled observed events in field experiments with similar external stresses and variations, the data are far from sufficient to allow definite conclusions. On the other hand, the results are encouraging, and more experiments along similar lines should be conducted. One of the difficulties with the work reported here is that, because of time restrictions, it was not possible to repeat each experiment enough to establish strict relationships.

For example, if the results of series 1 could be validated by sufficient repetition, then the effects of small environmental changes could be more readily evaluated. In this manner it might be possible to determine, for example, whether artificial circulation of a lake, with subsequent lowering of pH and elevation of nutrient concentrations, would be more likely to cause a shift from blue-green algae to green algae at one time in the history of an algal bloom than at an earlier or later point in that history. Such information would be of immense practical value and would be a step toward an ecological approach to lake restoration (Shapiro 1978).

The use of cyanophages is an attractive method for biological control, in that it is catalytic and avoids poisoning the general environment. The strong specificity of the cyanophages could provide selective control of blue-green algae, although development of resistant algal clones might be expected after large viral infections.

The need to understand the cause of algal blooms -- both biologically and chemically -- remains (Walsby 1970, Reynolds and Walsby 1975).

ACKNOWLEDGEMENTS

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III. DIRECT MANIPULATION OF ZOOPLANKTON POPULATIONS.*

The manipulation of herbivore abundance, as will be described later, can be done in a variety of indirect ways. However, work by Fritsch (1953) and Murphy (1970) indicated that a direct approach might be possible. Fritsch had found that additions of yeast to the _Chlamydomonas_ he was feeding to cultures of _Daphnia pulex_ had the effect of increasing the lifespan of the cladoceran. Investigating further, he was able to show that pantothenic acid, a constituent of yeast, had the same effect, and could, at a concentration of 200 ug/l, increase the life span of the _Daphnia_ by threefold. He also claimed that pantothenic acid increased egg production. Murphy's results with _D. pulex_ were somewhat different. He found that although the _Daphnia_ would survive on a diet of _Chlamydomonas_ and _Scenedesmus_, along with calcium acetate, bovine albumin and several antibiotics, they produced infertile eggs. However, addition of calcium pantothenate caused a very striking increase in the number of viable young, with the optimum concentration ranging from 0.2 to 2.0 mg/l. He did not show any effect on the life span, however.

Although both of these experimenters worked with less-than-natural conditions, Fritsch with _Chlamydomonas_ as the sole food, and Murphy with a mixture of algae and certain supplements, it seemed possible that panthothenic acid additions to a natural system might be one way to increase the _Daphnia_ population substantially. Accordingly, in July of 1976 we set our four enclosures in Little Lake Johanna a small lake just north of St. Paul, Minnesota. The enclosures were 1-meter diameter polyethylene bags sealed at the bottom and open at the top and holding approximately 1350 liters of lake water. The experiment ran from July 16 to September 9. Pantothenic acid was added to the bags once on July 16, as a solution of calcium pantothenate, to give final concentrations of 0.02, 2.0, and 20 mg/l respectively. The cladocerans were sampled periodically by vertical net tows with a plankton net. Results are shown in Tables 18 and 19.

It is clear that neither the _Daphnia_ nor the total cladocerans showed increases as a result of the pantothenic acid additions. In fact, it almost appears as though the additions reduced abundance of the animals. Our results may indicate that pantothenic acid is not required by _Daphnia_ under natural conditions. Certainly its effects, if any, were insufficient to warrant further study.

It should be mentioned that there are other chemical treatments that directly _reduce_ cladoceran abundance. For example, rotenone is known to kill many of the microcrustaceans when applied in concentrations used to eliminate fish (Hoffman and Olive, 1961; Kiser _et al_. 1963) (see section IV B for effects in Wirth Lake). However, when used, as in Minnesota, in the fall, its effects are probably gone by spring.

Pesticides also are very effective at eliminating, especially, cladocerans. This is particularly true for the organophosphorus pesticides, many of which have EC-50 values for _Daphnia pulex_ of less than 1 ug/l (Shapiro 1980). Pesticides may in fact be even more detrimental than this would indicate. Gliwicz (personal communication) is finding that concentrations of Lindane only about 1% as high as the EC-50 significantly reduce fecundity of _Daphnia_.

The possibility of adding zooplanktonic herbivores to a lake to increase their abundance directly does not appear to be really worthwhile, except perhaps under circumstances where all previously existing individuals have been killed by some substance such as rotenone. This has in fact been done. In 1950 the Canadian Wildlife Service treated a small lake in the Canadian

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*by Joseph Shapiro

Rockies with rotenone. One week later zooplankton were netted in nearby Moraine Lake and transported in milk cans to the treated lake and dumped in. Whether they survived is unknown. Under such circumstances the introduced individuals could act as an innoculum, but under "normal" circumstances it is doubtful whether one could add enough herbivores to affect the abundance meaningfully.

Furthermore, there is good evidence that the presence of a given zooplankter in a given natural system does not depend on whether or not the organism has been put into the system, but depends on whether or not the organism can survive in the system. An example is the chain of closed lakes in the Grand Coulee of western Washington State (Shapiro, unpub.). In this system, Lake Lenore had long contained two species of *Diaptomus* but, probably because of its greater salinity, nearby Soap Lake did not support members of this genus. However in the 1950s Soap Lake gradually became less saline because of leakage of gresh water into it from a large irrigation project. Accordingly, in the late '50s large populations of *Diaptomus* appeared in Soap Lake. Experiments in which Soap Lake water was concentrated an additional 10% showed that the organisms would not then live in it, substantiating the fact that it was the dilution that allowed the crustaceans to survive.

Another example in the same area is Lake Washington, which for reasons unknown has lately become inhabited by a sequence of species of *Daphnia*. All of them had been present in surrounding waters and therefore had the opportunity to become abundant in the lake, but hitherto had not done so (Edmondson, 1979).

In our own studies, Lake of the Isles (Section IV D) presents a good example. *Daphnia magna* is not normally looked upon as a lake species, and O'Brien (pers. comm.) feels that it cannot coexist with planktivorous fish. In winter 1976-77 Lake of the Isles suffered a winter-kill, and in 1977 *Daphnia magna* was found on several occasions.

Thus it appears as though efforts to manipulate herbivore populations directly are probably not likely to succeed. However, as will be shown below, in the following sections, great success can be expected from manipulations of the predators of herbivores.

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Table 18: Numbers of cladocerans* per net haul in enclosures treated with calcium pantothenate at the concentrations indicated.

Sample dates	0, Control	0.02 mg/l	2.0 mg/l	20 mg/l
July 16	1755	1528	4028	1200
July 19	2236	3094	3692	3450
July 30	2822	2154	3128	1443
August 2	5334	2926	3969	1346
August 6	5576	2393	3249	2172
August 29	3275	4879	3198	3795
September 9	2641	3264	4687	7332
$\bar{x}$ 7/30 - 9/9	3929	3123	3646	3217

*Cladocerans include *Daphnia*, *Bosmina*, *Ceriodaphnia*, *Diaphanosoma*, *Chydorus*, and *Scapholebris*

Table 19: Numbers of *Daphnia* per net haul in enclosures treated with calcium pantothenate at the concentrations indicated.

Sample dates	0, Control	0.02 mg/l	2.0 mg/l	20 mg/l
July 16	390	24	315	270
July 19	52	208	552	253
July 30	1054	329	1088	333
August 2	1155	152	294	137
August 6	1579	66	361	194
August 29	2095	1230	1784	1139
September 9	931	648	3397	2867
$\bar{x}$ 7/30-9/9	1362	485	1384	934

IV. MANIPULATIONS OF PLANKTIVOROUS FISH - EFFECTS ON ZOOPLANKTON AND PHYTOPLANKTON*

IV A. EXPERIMENTAL STUDIES

Our first studies of the effects on algae of manipulating populations of planktivorous fish were done in enclosures in two small bodies of water -- Lake Emily and Williams Pond -- both in St. Paul, Minnesota. These experiments showed that the effects of the fish were dramatic (Shapiro et al. 1975). We decided to extend the studies and examine the phenomenon in more detail in Pleasant Pond. We also attempted an experiment in Loch Loso, (Minneapolis) in which, after adding planktivores for a period, piscivores were added to reverse their effects. The results of these studies are presented below.

PLEASANT POND

Studies in this pond were done in 1975 and 1976. As two detailed papers describing the results are published (Lynch 1979,Lynch and Shapiro 1981) what follows is a summary of the material relevant to this report.

Pleasant Pond (about 0.25 hectares, mean depth 2.5 meters) is about 10 km north of St. Paul, Minnesota. The basin, excavated about 1965, has no surface inlet or outlet and lies in a sandy outwash plain of glacial origin surrounded by oak forest and secondary growth. Because of its shallowness the pond normally winter-kills and was devoid of fish when our work began. Three studies are described below -- one in which fish were added to enclosures, one in which fish and/or nutrients were added to enclosures, and one in which the whole pond was divided, with fish added to one half.

Enclosure Studies with Planktivorous Fish

On June 15, 1975, 12 1-m diameter 0.8 m deep polyethelene enclosures, closed at the bottom, were suspended in the pond and filled with surface water. Ten were stocked with five different densities of bluegill sunfish (*Lepomis macrochirus*) in duplicate, the remaining two serving as controls. Two of the fish enclosures failed to survive the experiment and fish escaped from two others. Thus results are available from only eight enclosures (Table 20). Furthermore, as the single fish in one of the enclosures died after five days, this enclosure was treated as another control. During the experiment which lasted until July 25, zooplankters were sampled weekly by vertical tows in the enclosures using a Wisconsin net with a 15 cm diameter opening and 64 um netting. Average coefficients of variation for four samplings on three occasions were, 0.36 for cladocerans, 0.45 for nauplii, 0.59 for copepods, and 0.59 for rotifers. All samples were fixed immediately with formalin/sucrose (Haney and Hall 1973). Phytoplankton samples taken from near the surface of the enclosures were fixed with Lugol's solution. Counts were made with a Wild inverted microscope and were converted to cell volumes using means of measurement of 10-50 individuals. Primary production was measured by the C-14 method with incubation at a depth of 0.5 meters for 24 hours. All incubations were on cloudless days. All oxygen and temperature measurements were made with a model 54 YSI meter. pH was determined in the laboratory with a Beckman meter. Total phosphorus, soluble reactive phosphorus, nitrate, nitrite, and alkalinity were determined according to Standard Methods (APHA 1971). Ammonia was determined as in Chaney and Marbach (1962).

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*by Michael Lynch and Joseph Shapiro

Table 20: Bluegill sunfish added and recovered from enclosures. Enclosures 1 and 7 were controls. Since its one fish only lived for 5 days, enclosure 2 is treated as a control.

	Added - 18 Jun		Recovered - 2 Aug	
Enclosure	Length (mm)	Wt (g)	Length (mm)	Wt (g)
2	76	7.8		
8	76	7.8	101	21.7
9	83	11.0	102	22.5
	70	4.8	80	9.8
3	108	23.2	108	24.3
	64	1.8	96	16.8
11	101	20.4	101	19.1
	87	14.0	87	10.2
	76	7.8	80	10.0
	75	7.8	75	6.4
6	114	26.2	114	24.9
	108	23.2	103	20.0
	102	20.4	102	19.0
	89	14.0	88	13.2
	79	9.2	84	9.9

Adding fish to the enclosures had a dramatic effect on the zooplankton community. As shown in Figure 62, three species completely disappeared from all fish enclosures -- *Daphnia pulex*, *Daphnia galeata mendotae* and *Chaoborus*. Three others appeared only in fish enclosures at the end of the experiment -- *Daphnia ambigua*, *Daphnia parvula*, and *Asplanchna priodonta*. While *Ceriodaphnia* increased at the lowest fish density, it remained in low abundance at higher fish densities. *Cyclops vernalis* appeared to be immune to fish predation, as was *Bosmina longirostris* which increased with intensity of fish predation. Several rotifers also increased with increasing predation (Fig. 63).

The effects of the fish treatments on the phytoplankton community were just as dramatic. Not only were the algal volumes in the bags with the fish about 16 times as great, on the average, as in the bags without fish, but there were great changes in species composition as well (Table 21). In the enclosures without fish the predominant algae were *Cryptomonas*, *Ceratium*, *Closterium*, and *Penium*. At the lowest level of fish predation, the gelatinous form *Oocystis* became dominant along with *Scenedesmus*, and, as fish predation increased, filamentous blue-greens became more predominant in the community with *Aphanizomenon flos-aquae* making up 72% of the volume in the enclosure with 5 fish. Indeed the density of filamentous blue-greens (*Anabaena* and *Aphanizomenon*) shows a good inverse relationship to the abundance of *Daphnia* plus *Ceriodaphnia* (Fig. 64). The implication is that there is a threshold in density of these cladocerans below which such algae can escape grazing. The abundance of flagellates shows a similar relationship to the density of *Daphnia* and *Ceriodaphnia*, (Fig. 65), but the flagellates continue to be an important part of the algal community even at the highest concentrations of the cladocerans. Presumably this group of algae is able to maintain itself under intense grazing conditions (Fott, in press).

There is little doubt that the changes in zooplankton abundance resulted from predation by the fish, and the relationships in Figures 64 and 65 suggest strongly that algal changes were a result of the zooplankton changes i.e. as the major herbivores were eliminated the algal biomass became greater.

Certainly there is no evidence that phytoplankton alterations resulted from changes in nutrients because of the treatments. Table 22 shows that total phosphorus was at much the same concentration in all enclosures and the pond, and in fact averaged about 10% greater in the fishless enclosures than in those with fish. Neither ammonia nor nitrate became undetectable and the differences did not seem systematic. The high concentrations of PO4-P in the pond and fishless enclosures presumably result from the rapid turnover of the algal population by the abundant grazers and the inability of the few remaining algae to remove all the available phosphate from solution.

Enclosure Studies with Planktivores and/or Nutrients

Despite the fact that there was no evidence that the effects of the fish treatments were related to nutrients, a second series of experiments was done in 1976 to test the idea directly. This experiment, from May 29 to July 15, was begun with twelve 1.8 m deep enclosures, six containing two small (60-70 mm) *Lepomis macrochirus* each, and six without fish. Three levels of enrichment were used so that for enclosures with and without fish two were left as controls (LN), two received 10 ug/l phosphorus plus 110 ug/l nitrogen per week (MN), and two received 20 ug/l phosphorus and 330 ug/l nitrogen per week (HN). Nutrients were added as solutions of KH2PO4 and KH4NO3 and

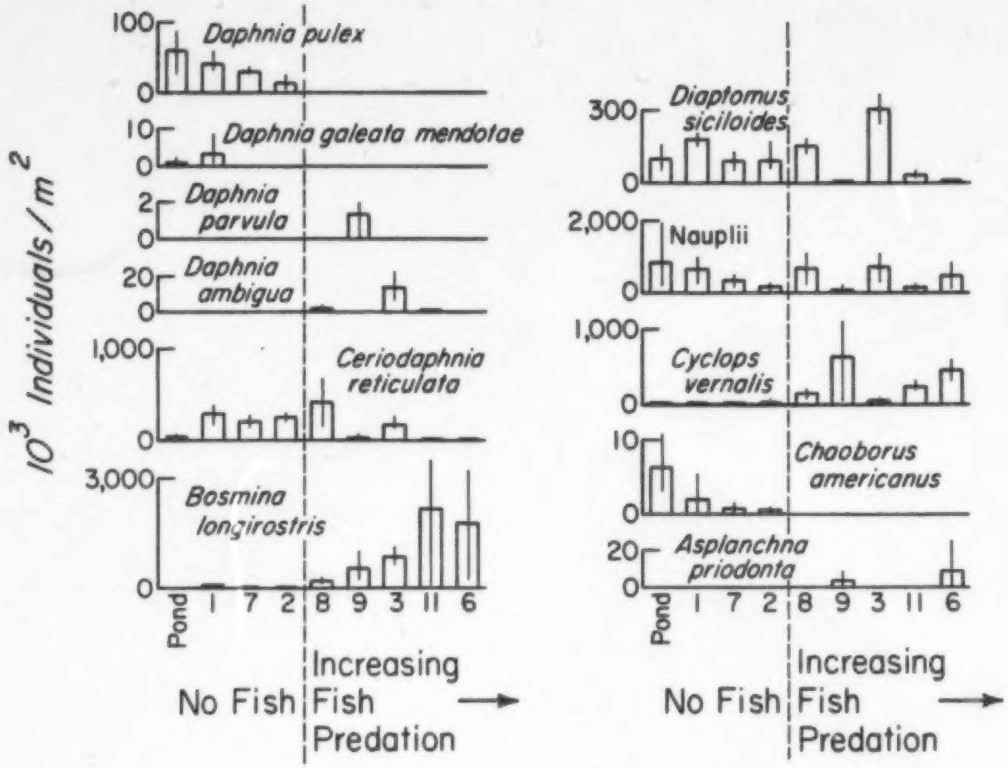

Fig. 62. Mean and range for abundance of crustaceans, Chaoborus and Asplanchna for last three sampling dates of enclosure experiment.

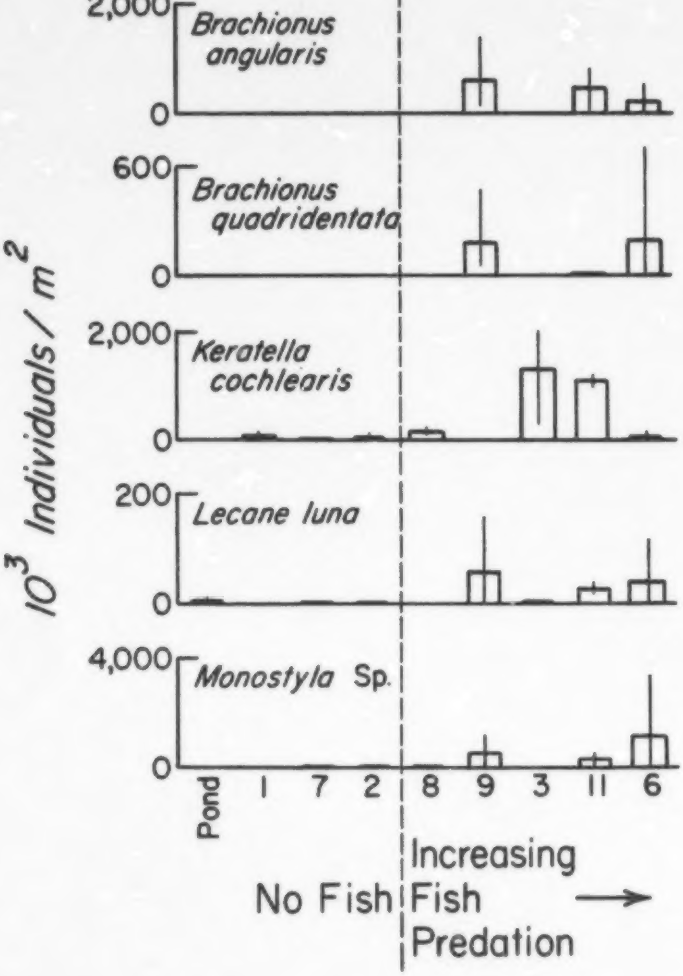

Fig. 63. Mean and range for abundance of predominant rotifers for last three sampling dates of enclosure experiment.

Table 21: Effects of increasing levels of fish predation on mean total biomass ($10^5/um^3/ml$), and proportional abundance of five dominant species. (Data are means for the last 3 sampling dates.)

NO FISH

POND	15.91 ± 3.59
Cryptomonas tetrapyrenoidosa	.50
Ceratium hirundinella	.18
Heterochromonas globosa	.14
Cryptomonas marsonii	.07
Botrydiopsis arhiza	.05

ENCLOSURE 1	6.01 ± 3.57
Ceratium hirundinella	.46
Cryptomonas tetrapyrenoidosa	.21
Heterochromonas globosa	.10
Closterium moniliferum	.06
Cosmarium sexnotatum	.04

ENCLOSURE 7	4.97 ± 2.00
Cryptomonas tetrapyrenoidosa	.43
Closterium moniliferum	.31
Ceratium hirundinella	.11
Cosmarium sexnotatum	.05
Aphanizomenon flos-aquae	.02

ENCLOSURE 2	8.77 ± 1.23
Penium sp.	.27
Cryptomonas tetrapyrenoidosa	.26
Closterium moniliferum	.22
Ceratium hirundinella	.16
Cosmarium reniforme	.02

FISH ENCLOSURES

ENCLOSURE 8 (1 fish)	114.73 ± 4.21
Oocystis lacustris	.52
Scenedesmus armatus	.20
Sphaerocystis schroeteri	.08
Ankistrodesmus falcatus var. mirabilis	.03
Schizochlamys planctonica	.03

ENCLOSURE 9 (2 fish)	106.60 ± 37.85
Anabaena circinalis	.47
Cryptomonas tetrapyrenoidosa	.37
Cosmarium sexnotatum	.04
Pediastrum boryanum	.03
Schizochlamys planctonica	.02

ENCLOSURE 3 (2 fish)	83.41 ± 36.18
Oocystis lacustris	.46
Anabaena circinalis	.09
Cosmarium sexnotatum	.08
Heterochromonas globosa	.07
Sphaerocystis schroeteri	.07

ENCLOSURE 11 (4 fish)	88.78 ± 26.37
Anabaena circinalis	.64
Cryptomonas tetrapyrenoidosa	.30
Aphanizomenon flos-aquae	.02
Treubaria crassispina	.01
Sphaerocystis schroeteri	.01

ENCLOSURE 6 (5 fish)	308.05 ± 248.80
Aphanizomenon flos-aquae	.72
Heterochromonas globosa	.08
Anabaena circinalis	.07
Sphaerocystis schroeteri	.04
Cryptomonas tetrapyrenoidosa	.02

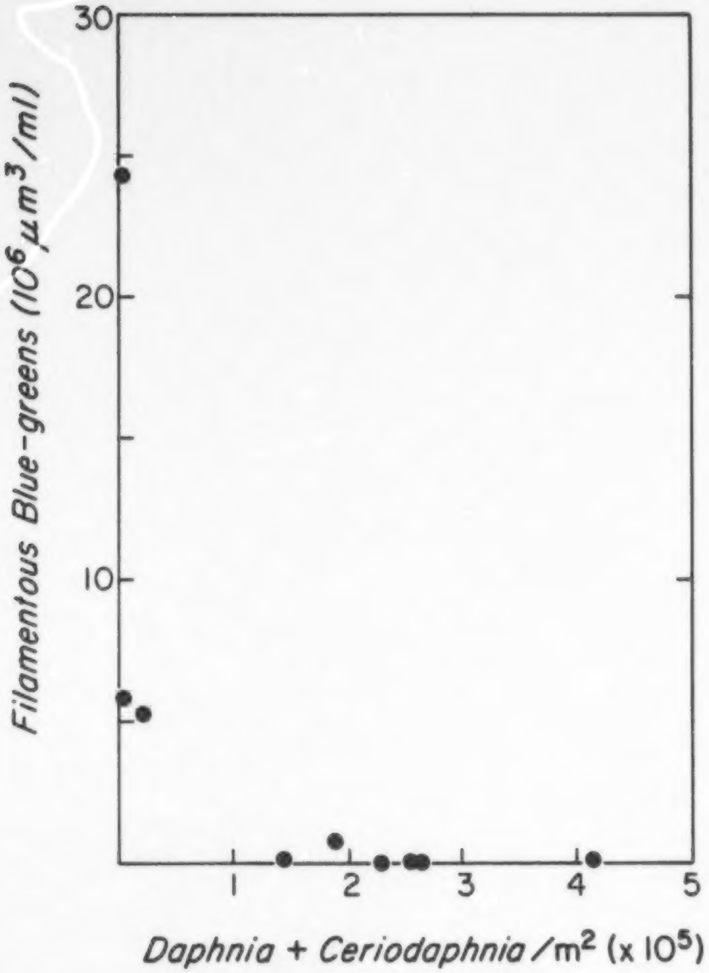

Fig. 64. Mean density (last 3 sampling dates) of filamentous blue-greens vs. mean density of Daphnia and Ceriodaphnia in enclosures.

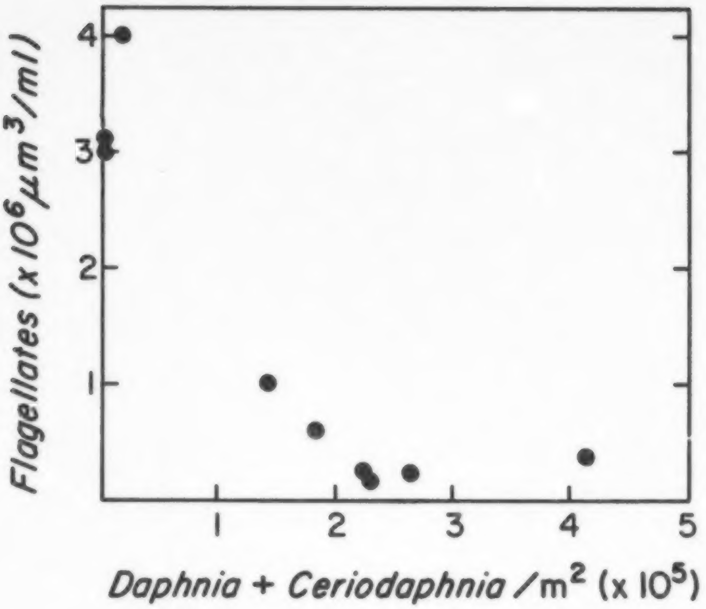

Fig. 65. Mean density (last 3 sampling dates) of flagellates vs. mean density of Daphnia and Ceriodaphnia in enclosures.

Table 22: Chemical conditions in enclosures with and without fish (means for last 3 sampling dates).

	CONTROLS				FISH ENCLOSURES				
Enclosure #	POND	1	7	2	8	9	3	11	6
Number of fish	--	0	0	0	1	2	2	4	5
Total Phosphorus (ug/l)	160	100	119	134	100	121	106	92	128
PO_4 - P (ug/l)	84	23	38	43	4	3	4	2	9
NH_3 - N (ug/l)	26	26	31	38	2	7	20	48	0
NO_2 - N + NO_3 - N (ug/l)	14	13	13	19	30	37	12	26	30
pH	8.02	8.00	8.10	8.00	8.18	9.00	8.26	8.41	8.28

stirred in after weekly samples had been taken. The N/P ratio was chosen to equal that of the pond at the onset of the experiment. Two enclosures failed to survive and three had only one fish left alive at the end of the experiment. Nutrient concentrations in the enclosures are shown in Table 23.

It is clear from Figure 66 that nutrient enrichment was not the major determinant of zooplankton community composition. In all enclosures without fish Daphnia pulex was the dominant herbivore with Ceriodaphnia, Cyclops, nauplii and rotifers remaining rare. In the enclosures with fish, Cyclops and nauplii appear to have increased at the medium levels of enrichment, and rotifers increased at medium and high levels. However the presence of the fish resulted in the removal of Daphnia as well as reduction in the abundance in Diaptomus at all levels of enrichment.

As in the first experiment there were accompanying changes in the abundance and community structure of the algae (Table 24). On the average, algal volumes in the fish enclosures were 2.5 times as high as in those with no fish but in neither case did the algal abundance bear a relationship to the nutrient status of the enclosures.

In the enclosures without fish, enrichment caused increases in Cosmarium and in Chroococcus, two algae of relatively poor nutritional quality for herbivores. In the presence of the fish, desmids increased at the higher nutrient levels. Qualitative differences between the enclosures with fish and those without were not as striking as in the first enclosure experiment. Indeed there were more blue-greens in the enclosures without fish than in those with fish, but as noted above these were mainly Chroococcus, a gelatinous blue-green. Perhaps the most striking effect was the appearance of Ceratium in the enclosures with fish.

As in the first experiment, then, the fish removed the larger herbivores and the increase in algal biomass in the presence of fish was apparently not nutrient-related. In the second experiment, the increase in algal volume resulting from fish addition was smaller than in the first experiment. This was in agreement with the fact that in the second case enclosures without fish contained only about one-fourth as many large herbivores as in the first experiment.

Pond Separation Experiment

To test the effects of adding fish on a larger scale than hitherto, on April 27, 1976, Pleasant Pond was divided in two. We used a double curtain of nylon-reinforced polyethylene sheeting weighted firmly into the sediments with steel chain and held about 25 cm above the water surface by a cable stretched above the pond, and by styrofoam floats attached to the upper edge of the curtain. The north half (PPN) was stocked by the Minnesota Department of Natural Resources on May 5 with 10,000 walleye fry (Stizostedion vitreum). Despite our seining efforts throughout the summer we neither saw nor captured any of these fish. However, in early October the DNR was able to capture 306 fish (total weight 7.7 kg) after two days of intensive trap netting. Thus it appears that most of the fish had not survived. Fortunately on June 5 several mature fathead minnows (Pimephales promelas) were sighted in the southern half of the pond (PPS) and by June 13 schools of newly-hatched minnows were visible from shore. On August 3 sampling of a 19 m2 area with a 3 mm mesh seine yielded 500 minnows of average length four cm. The origin of these fish is unknown but since they were present in PPS only, and since PPN had very few walleye, we merely redesignated the ends of the ponds so that PPN was the fishless control and PPS was the end with fish.

As we had expected the zooplankton population responded essentially as

Table 23: Chemical conditions, algal biomass, and photosynthetic rates in control and enriched enclosures with and without fish.

Enclosure #	1	7	3	9	5	2	8	4	6	12
Number of fish	0	0	0	0	0	2	2	2	2	2
Nutrient regime	LN	LN	MN	MN	HN	LN	LN	MN	HN	HN
Total Phosphorus (ug/l)	34	24	60	44	58	29	44	61	108	108
PO_4 - P (ug/l)	2	4	5	3	5	2	3	18	14	10
NH_3 - N (ug/l)	40	23	56	58	76	16	59	96	111	70
NO_2 - N + NO_3 - N (ug/l)	6	22	76	34	196	6	8	36	186	134
pH	9.5	9.0	9.4	9.7	9.9	9.2	9.1	9.7	9.8	10.0
mg C fixed/liter/day	.07	.09	1.11	.07	.06	.36	.15	2.02	6.56	2.35
10^{-4} pg C fixed/u^3 phytoplankton/day	0.8	0.8	1.6	0.3	0.4	0.7	0.3	4.40	13.48	1.95
$10^7 u^3$ phytoplankton/ug total phosphorus	2.5	4.9	11.5	5.1	2.4	17.6	11.7	7.5	4.5	11.2

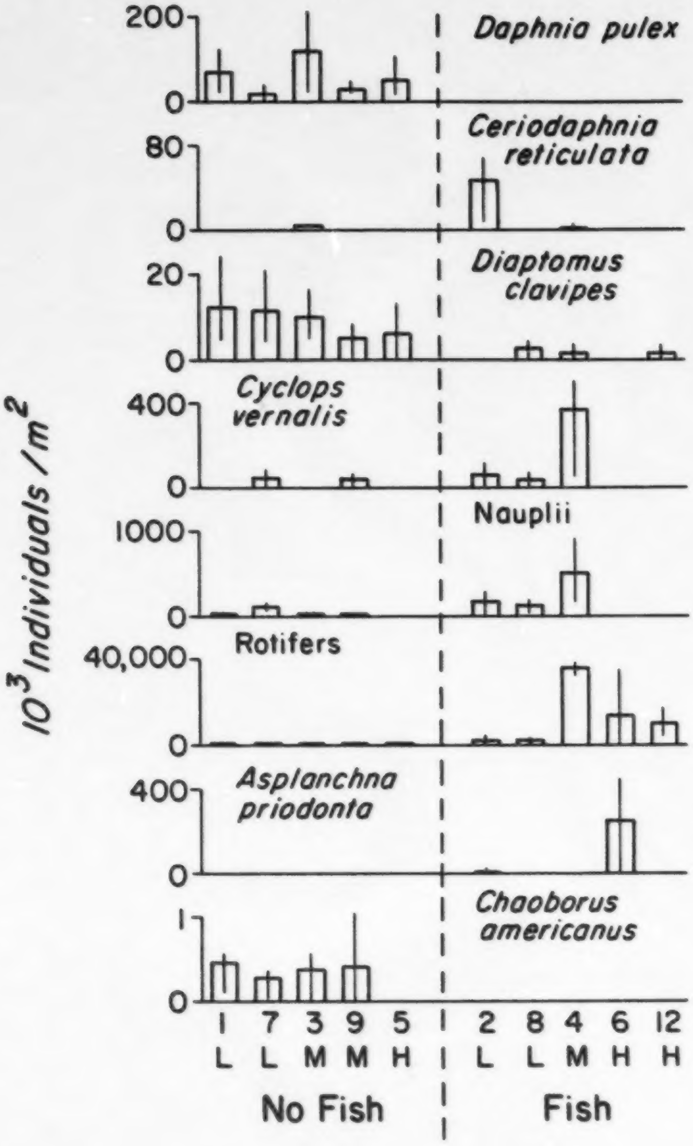

Fig. 66. Mean and range (last 3 sampling dates) for abundance of zooplankton species in enclosures at different nutrient concentrations. Nutrient levels are L - low (controls); M - medium; H - high.

Table 24: Effects of fish predation on mean total phytoplankton biomass (10^5 um^3/ml) and proportional abundance of five dominant species at different levels of enrichment. (Data are means for last 2 sampling dates.)

NO FISH		FISH ENCLOSURES (2 fish each)	
ENCLOSURE 1 - LN	8.55	ENCLOSURE 2 - LN	51.05
Closterium moniliferum	.62	Staurastrum paradoxum	.57
Staurastrum paradoxum	.13	Ceratium hirundinella	.29
Ulothrix oscillarina	.10	Cosmarium sexnotatum	.05
Chroococcus dispersus	.10	Cyclotella sp.	.03
Heterochromonas globosa	.01	Gloecystis vesiculosa	.01
ENCLOSURE 7 - LN	11.71	ENCLOSURE 8 - LN	51.55
Rhodomonas minuta	.35	Ceratium hirundinella	.43
Staurastrum paradoxum	.31	Closterium moniliferum	.20
Chroococcus dispersus	.12	Cyclotella sp.	.10
Pediastrum boryanum	.04	Cosmarium sexnotatum	.08
Gloeocystis vesiculosa	.04	Staurastrum paradoxum	.06
ENCLOSURE 3 - MN	69.06	ENCLOSURE 4 - MN	45.88
Chroococcus dispersus	.60	Staurastrum paradoxum	.77
Cosmarium reniforme	.17	Cosmarium sexnotatum	.10
Cosmarium sexnotatum	.10	Ceratium hirundinella	.08
Aphanizomenon flos-aquae	.05	Chroococcus dispersus	.02
Sphaerocystis schroeteri	.02	Chlamydomonas sp. 1	.01
ENCLOSURE 9 - MN	22.38	ENCLOSURE 6 - HN	48.67
Chroococcus dispersus	.92	Staurastrum paradoxum	.61
Straurastrum paradoxum	.05	Cosmarium sexnotatum	.33
Ulothrix oscillarina	.01	Chroococcus dispersus	.03
Heterochromonas globosa	.01	Pediastrum boryanum	.01
Oscillatoria tenuis	<.01	Trachelomonas sp. 1	.01
ENCLOSURE 5 - HN	13.88	ENCLOSURE 12 - HN	120.38
Chroococcus dispersus	.49	Scenedesmus denticulatus	.76
Cosmarium sexnotatum	.38	Staurastrum paradoxum	.17
Staurastrum paradoxum	.05	Scenedesmus quadricauda	.02
Oscillatoria tenuis	.03	Cosmarium sexnotatum	.02
Ulothrix oscillarina	.01	Oscillatoria tenuis	.01

it had in the enclosures (Fig. 67). By the end of June the fathead minnow population had become well established and the zooplankton populations in the two halves of the pond began to diverge. In Pleasant Pond South, *Daphnia pulex* was eliminated by the end of July, and *Ceriodaphnia* and *Diaptomus clavipes* remained very rare. At the end of summer the two small daphnids, *D. ambigua* and *D. parvula* appeared as they had in the "fish" enclosures in experiment 1. Nauplii were unaffected by the predation but rotifers became very abundant. In PPN, *Daphnia pulex* became very abundant through July then gave way to *Ceriodaphnia*. Rotifer numbers remained low.

Coincident with these differences in zooplankton there were large differences in the algal biomass and species composition. Until mid-June (Table 25) the two halves of the pond maintained essentially equal concentrations of algae. However, from June 25 on, algal volumes in the south end were greater, and for the period June 25 to July 23 averaged 5 times as great. The picture is complicated by the fact that total phosphorus in both halves of the pond increased until mid-June, remained high, and fell again in August (Table 25). The reasons for these increases are unknown. 1976 was a particularly dry year and part of the increase may be a consequence of evaporation. Certainly there was no runoff from June through August. Also as the ponds stratified in 1976 and the bottom waters became anoxic, sediment P release may have contributed. Furthermore the minnows in the south half may also have increased phosphorus concentrations there through excretion, as when the zooplankters became very scarce the minnows began to feed on the sediments. In the northern half this explanation was not tenable but dense stands of the macrophyte *Callitriche* developed, and they may have transferred nutrients from the sediments to the water.

In any event it is clear from Table 25 that increases in total phosphorus notwithstanding, from June 25 to early August phytoplankton/total P ratios were higher in PPS, averaging 3.4 times as great as those in PPN. The low ratios and low algal abundances in the north end were not a result of nutrient limitation. As shown in Table 25 from June 12 to July 23 specific rates of carbon fixation in the north end were equal to or even greater than those in the south end.

Composition of the algal community in both ponds was similar through May consisting mainly of dense populations of flagellates (*Chlamydomonas* spp., *Cryptomonas obovata*, and *Phacus* spp.). During June both pond halves developed blooms of *Aphanizomenon flos-aquae*. In the south half the bloom crashed and was replaced in July by a dense bloom of the green alga *Ankistrodesmus*, following which *Oscillatoria tenuis* and *Anabaenopsis* became abundant through August. In the north half the *Aphanizomenon* bloom collapsed, recovered, and finally disappeared by late-July. During August a variety of forms were present including flagellates, diatoms, *Sphaerocystis* and the filamentous *Oscillatoria* and *Ulothrix*, but total biomass remained low.

Thus as in the enclosure experiments, addition of fish had the effect of eliminating the larger herbivores and increasing the algal biomass many-fold. Nutrients did not seem to be involved as rates of primary production were equally high in the pond halves with and without fish. Furthermore in all cases increased fish predation led to increased algal biomass despite greater abundance of small herbivores.

LOCH LOSO

Loch Loso is a small body of water consisting of two interconnected ponds having a total area of about 1/2 hectare. The ponds are on the grounds

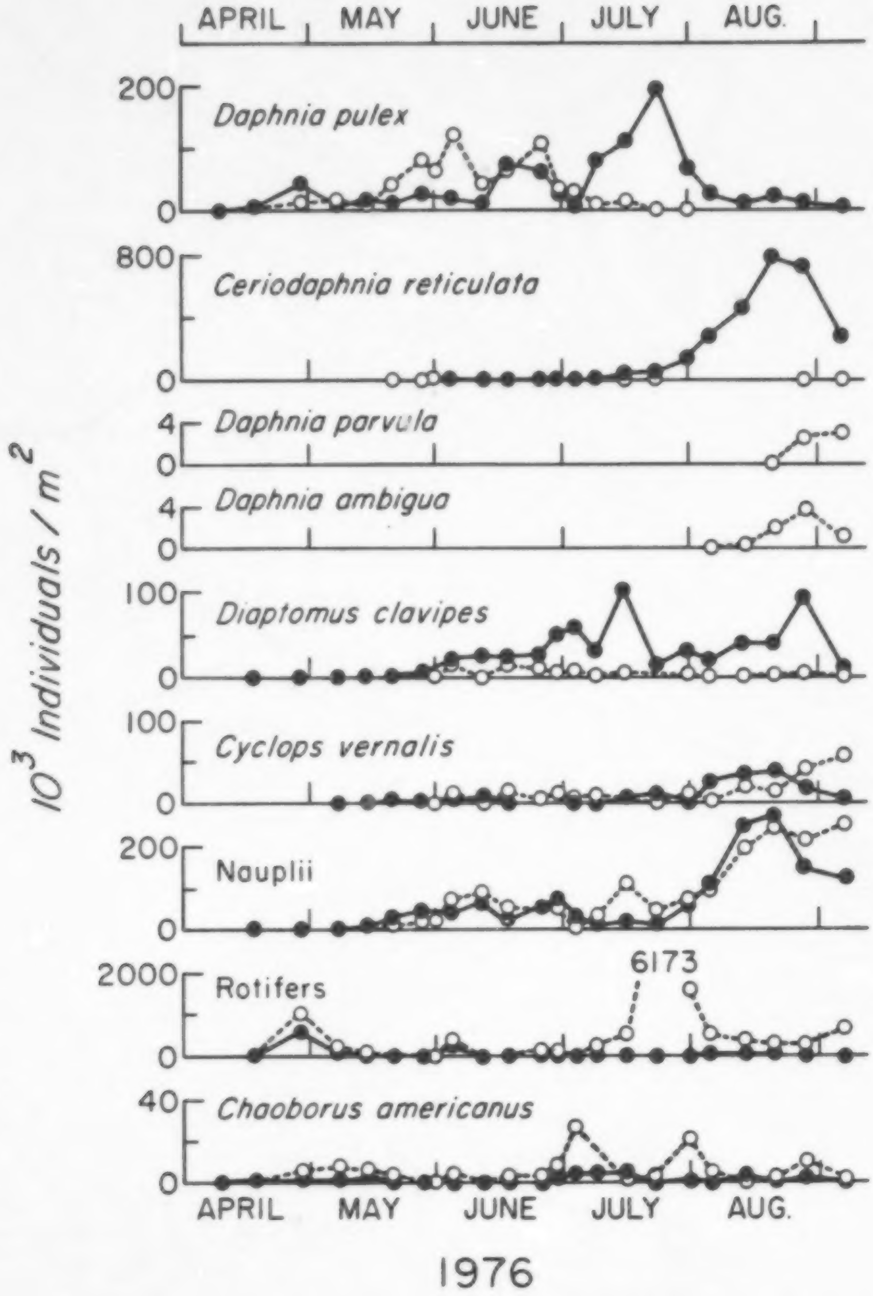

Fig. 67. The 1976 abundances of main zooplankton species in Pleasant Pond North having no fish (solid lines) and Pleasant Pond South in which fathead minnows began to appear in June (dashed lines).

Table 25: Total P, phytoplankton, community and specific photosynthetic rates and biomass/TP ratios in Pleasant Pond, 1976.

	TP mg/m^3		phytoplankton 10^7 um^3/l		mg C fixed/l/dy		10^{-4} pg C fixed/um^3 phytoplankton/dy		10^7 um^3 phytoplankton/ug TP	
Date	PP N	PP S	PP N	PP S	PP N	PP S	PP N	PP S	PP N	PP S
May 14	33	26	83	55	0.05	0.06	0.7	1.0	2.5	2.1
May 27	43	47	52	47	0.06	0.10	1.2	2.2	1.2	1.0
June 12	120	71	288	220	1.40	0.86	4.8	3.9	2.4	3.1
June 25	119	193	762	4130	1.15	0.87	1.5	0.2	6.4	21.4
July 9	109	119	207	690	0.74	0.93	3.5	1.4	1.9	5.8
July 23	102	157	61	345	0.23	0.92	3.7	2.7	0.6	2.2
August 20	12	39	254	332	0.11	1.66	0.4	5.0	21.2	8.5

of Hennepin Community College in Minneapolis and are used to collect storm runoff. As a result they are productive. The larger half (HCC1) encompasses about 2/3 of the total area, and has a maximum depth of 3 meters, The smaller half (HCC2) has a mean depth of about 2 meters. Being shallow, the ponds normally winter-kill. As a result when we first inspected them late in 1974 the ponds had substantial populations of *Daphnia pulex*. They also had a so-called "flake" or "grass blade" bloom of *Aphanizomenon*.

Our intention was to divide the ponds early in 1975, stock the larger half with planktivorous fish (using the other as a control), and determine the effects of the fish on the zooplankton and algae. Then we proposed to add piscivorous fish to the larger half early in 1976 and follow the results for a year more. This plan was followed but not exactly as we had hoped. 1975 was a wet year, and although we did separate the ponds and add planktivores to HCC1, (350 mature perch and 100 bluegill sunfish) the ponds soon became joined as a result of greatly increased water levels and remained so until July until after the fish reproduced. Therefore in late 1975 we removed the barrier and allowed the fish to equilibrate to both sides. On April 9, 1976, the ponds were divided again and 34 northern pike (*Esox lucius*) about 13 inches long were added to HCC1. However the planktivorous fish had become so abundant that the predators had little effect and even trapping fish from HCC1 and placing them in HCC2 did not lower the planktivore abundance in HCC1 low enough to allow *Daphnia* to reoccur.

The results of periodic vertical hauls for zooplankton are shown in Tables 26 and 27. *Daphnia pulex* was very abundant in both sides of the pond, in 1975 from March to early August in HCC1, and in 1975 from March to late August in HCC2 (Figs. 68 and 69). Smaller cladocerans such as *Bosmina* and *Chydorus* were relatively rare. In both halves *Diaptomus clavipes* were present early but gave way in June to large populations of *D. siciloides*. Rotifers were rare throughout the whole period. Clearly the planktivorous fish had little effect in 1975. However by 1976 the picture was different (Tables 26 and 27, Figs. 68 and 69). *D. pulex* was completely absent from HCC2 and was found at low concentrations in HCC1 on only two dates. *Bosmina* which had been rare in 1975, became abundant in July and August, and *Chydorus sphaericus* became the dominant cladoceran. *Diaptomus clavipes* was absent and *D. siciloides* became abundant in both parts of the pond. In contrast to 1975, rotifers became extremely abundant. The shift from *D. clavipes* to *D. siciloides* in 1976 may have resulted from predation by the planktivores, and the brief appearance of *D. pulex* in HCC1 in 1976 may have indicated the effect of the northern pike. However, it is clear that in this system, as opposed to Pleasant Pond and the enclosure systems, there was a lag of about a year in the effect of the planktivore introduction. The effect of the piscivore introduction could not be studied further in 1977 as a nearly complete fish-kill occurred in 1976-77.

Phytoplankton response to the zooplankton was as expected. In 1975, except for periods when *Aphanizomenon flos-aquae* formed "flake" blooms, chlorophyll concentrations were very low (Fig. 70 and Table 28). They were not low for lack of nutrients (Table 28) and in fact only on June 26 when chlorophyll concentrations were relatively high were there any indications that available nitrogen might be low enough to be limiting. Rather, the high concentrations of free phosphate, as in the enclosures in Pleasant Pond (above) implicate the high zooplankton grazing rate as the reason for the sparse algae. As a result of the low algal populations Secchi disc transparency (Figures 71 and 72 and Table 28) was high and remained so even on June 26 1975 when *Aphanizomenon* was present in flake form. Chlorophyll samples were collected in 1976 but lost before analysis. However,

Table 26: Zooplankton (thousands of individuals/m^2) in vertical net hauls in HCCl, 1975 and 1976.

	1975 March			April			May					June			
	4	11	26	2	9	25	2	7	14	23	28	6	15	20	26
Bosmina longirostris													.994	3.82	
Ceriodaphnia quadrangula													2.39		.866
Chydorus sphaericus						.303						11.4		1.28	5.20
Daphnia pulex	1.00	2.86	5.23	2.00	.697	2.73	12.1	86.2	154	18.7	35.8	81.3	21.9	19.1	31.2
Cyclops vernalis	.061	.636	.224	.333	.697	.606	.187			8.11	6.97	23.5	19.9	28.0	43.3
Mesocyclops edax															
Diaptomus clavipes	3.33	7.32	.299	.333	.460		.187			1.84	7.43	.713	1.99		.866
Diaptomus siciloides												8.56	33.8	36.9	58.9
Nauplii			.348		.697	1.21	.374		4.14	52.3	27.4	20.7	70.5	121	104
Asplanchna priodonta															
Brachionus angularis															
Brachionus calycifloris															
Brachionus platicatilis															
Brachionus quadridentata															
Colurella sp.	.667														
Epiphanes brachionus															
Filinia sp.															
Kellicottia longispina					.232										
Keratella cochlearis										.369	.929				
Keratella quadrata	.333	1.27							1.38	3.64	3.25	.713			
Keratella vulga															
Lecane luna										.309					
Monostyla sp.													1.99		
Mytlina sp.															
Platyias sp.															
Polyarthra sp.															
Synchaeta sp.															
Chaoborus													.398		

Table 26 Continued

	1975												1976					
	July				Aug.				Sept.		Oct.	Dec.	Jan.		Feb.	April		
	3	11	18	25	1	12	19	27	10	17	1	12	12	23	6	9	17	28
B. longirostris		1.49			3.39													
C. quadrangula	.978			.994														
C. sphaericus	3.91	2.98					37.7	283	2134	2151	711	8.80		4.28		2.51	6.21	17.3
D. pulex	6.85	53.7	53.2	12.9	15.8					5.34								
C. vernalis	39.1	41.8	136	1.99	2.26	25.4	25.9	48.7	63.3	48.1	22.4	2.93						5.77
M. edax																	8.27	5.77
D. clavipes	1.96	16.4	5.21	3.98	3.39													
D. siciloides	18.6	28.3	8.34	15.9	20.3	13.9	40.1	81.9	240	336	183	20.5	4.62	188	40.7	12.5	12.4	326
Nauplii	47.9	79.0	45.9	4.97	23.7	41.6	58.9	345	538	395	58.2		4.62	8.56		7.53	352	335
A. priodonta																		
B. angularis														8.56	7.41	5.02	168	317
B. calycifloris								42.0	583	10.7								
B. platicatilis																		
B. quadridentata					11.3													
Colurella sp.																		
E. brachionus												370	1055	711	411	547	3756	20.2
Filinia sp.																		
K. longispina																		
K. cochlearis							2.36					202	85.4	8.56	11.1			5238
K. quadrata													16.2	17.1	7.44		2.07	
K. vulga																		
L. luna	.978		1.04															
Monostyla sp.				.994														
Mytilina sp.																		
Platyias sp.																		
Polyarthra sp.												522	3606	2243	1019	1556	3822	20.2
Synchaeta sp.																		
Chaoborus																		

Table 26 Continued

	1976 May 7	14	20	27	June 4	11	17	25	July 3	8	16	22	30	Aug. 5	13	20	27
B. longirostris							3.18		6.93	10.2	207	283	272	743	3141	1371	4525
C. quadrangula								4.62	6.93	12.3	2.09						
C. sphaericus		2.45	2.24	8.29	7.50	11.8	20.6	126	194	67.5	29.3	23.8	15.2	7.94			
D. pulex								1.54		2.04							
C. vernalis	10.7	2.45	2.24														2.36
M. edax			2.24			18.5	23.8	26.2	194	161	205	188	111	36.5	126	91.0	84.8
D. clavipes																	
D. siciloides	243	258	204	82.9	5.63			16.9	8.66	14.3	10.5	9.14	3.37	1.59			2.36
Nauplii	681	363	505	451	91.9	205	197	180	355	472	310	283	104	105	81.8	203	292
A. priodonta	331	88.3				15.2	194	120	15.6			3.66					
B. angularis							127	877	528	511	425	314	10.1	7.94	3.99	6.21	2.36
B. calyciflorus																	
B. platicatilis						80.8											
B. quadridentata	64.1	29.5			41.3	6.73											
Colurella sp.						182											
E. brachionus																	
Filinia sp.	34.7	17.2	4.47								2.09				9.98		
K. longispina																	
K. cochlearis		2.45	11.2	852	6640	1542	1585	114	50.2	77.7	71.1			71.4	53.9	8.27	
K. quadrata	5.34	7.36	6.71	19.4	33.8	3.37	20.6	26.2	8.66	10.2							2.36
K. vulga					5.63	50.5	1.59	95.4	575	613	3925	241	226	133	216	314	238
L. luna																	
Monostyla sp.																	
Mytilina sp.							4.76	52.3	6.93		6.28		1.68				2.36
Platyias sp.							39.7	782	1510	16.4	2.09			1.59			
Polyarthra sp.	8649			5.53			316	459	53.7	4.09		1.83		1.59			
Synchaeta sp.							71.4										
Chaoborus																	

Table 27: Zooplankton (thousands of individuals/m^2) in vertical net hauls in HCC2, 1975 and 1976.

	1975 March			April			May					June			
	4	11	26	2	9	25	2	7	14	23	28	6	15	20	26
Alonella sp.															
Bosmina longirostris			.030												
Chydorus sphaericus				.020	.247							1.92	4.30	6.83	20.2
Daphnia pulex	7.15	5.09	.636	.783	1.98	1.01	2.43	61.5	98.2	19.1	101	20.2	34.4	44.4	21.4
Cyclops bicuspidatus													2.15		
Cyclops vernalis		.314	.030	.040	.247		.040		1.09	2.67	3.32				
Mesocyclops edax												.962	1.07	8.54	10.7
Diaptomus clavipes	2.23	1.82	.091	.040			.040			.222	1.42				
Diaptomus siciloides													1.07	3.42	1.19
Nauplii			.030			.673	.747	.586	2.55	22.2	12.8	53.9	85.9	49.5	34.4
Asplanchna priodonta															
Brachionus angularis															
Brachionus calycifloris															
Brachionus quadridentata															
Filinia sp.															
Keratella cochlearis	.447						.187				.475				
Keratella quadrata	2.23	.364	.061	.020			.187		2.18	2.89	1.42	1.92			
Keratella vulga															
Lecane luna													2.15		
Mytilina sp.													1.07		
Platyias sp.															
Polyarthra sp.															
Synchaeta sp.															

Table 27 Continued

	1975 July				Aug.				Sept.		Oct.	Dec.	1976 Jan.		Feb.	April		
	3	11	18	25	1	12	19	27	10	17	1	12	12	23	6	9	17	18
Alonella sp.																		
B. longirostris																		
C. sphaericus	46.5		2.15			6.93	66.7	20.6	280	751	381	37.5	26.6					2.20
D. pulex	5.05	116	46.2	57.3	44.4	100	44.4											
C. bicuspidatus																		
C. vernalis					2.70													1.47
M. edax	8.08	46.3	4.29	3.58	5.39	5.20												
D. clavipes																		
D. siciloides	5.05	16.0	4.29	7.17	13.5	34.6	22.2	3.44	14.7	112	102	9.86	1659	7.65	12.7	3.27		8.81
Nauplii	135	242	44.0	74.1	6.73	31.2	62.9	13.8	88.3	138	125	9.86					29.2	17.6
A. priodonta																	31.5	103
B. angularis												5.92						
B. calyciflorus																		2.94
B. quadridentata									243	32.9								
Filinia sp.												834	2260	1170	864	191	2492	13.2
K. cochlearis												70.9	26.6					
K. quadrata										6.59		5.92	16.6	3.82				32.3
K. vulga													3.32	22.9	2.55			
L. luna																		
Mytilina sp.																		
Platyias sp.																		
Polyarthra sp.												103	3253	1294	1048	177	7017	5672
Synchaeta sp.											15.9							23.5

Table 27 Continued

	1976																
	May				June				July					Aug.			
	7	14	20	27	4	11	17	25	3	8	16	22	30	5	13	20	27
Alonella sp.											10.8	1.65	29.6	7.86		1.83	3.08
B. longirostris												6.59	6.73	2.62	16.7	1.83	23.1
C. sphaericus	1.66	4.40	1.78	2.14		18.2	293	127	164	42.7	195	36.2	1.35	5.24		1.83	4.62
D. pulex																	
C. bicuspidatus																	
C. vernalis			12.5	8.56		4.98	3.01	3.90		3.56							
M. edax							6.01	33.1	77.3	90.7	152	219	64.6	128	80.2	85.9	159
D. clavipes																	
D. siciloides	49.8	7.34	1.78	4.28	5.10	11.6	7.52	5.84	1.21	3.56	14.4	1.65	5.39	7.86		1.83	
Nauplii	49.8	116	51.6	64.2	125	87.9	176	171	485	493	1053	196	246	315	410	583	363
A. priodonta	9.95		16.0	139	1277	662	21.0	19.5		1.78							
B. angularis				92.0								11.5	8.08	115	8.36		
B. calyciflorus	9.95	33.7	8.90	51.4	828	13.3											
B. quadridentata							263	5.84	3.03				21.5	21.0		11.0	27.7
Filinia sp.	8.30	98.3	171	21.4	12.7			1.95							1.67		
K. cochlearis		51.3	1335	1600	449	28.2	76.7	27.3	1.52	1.78						1.83	
K. quadrata	254	3192	1808	2525		3.32	1.50		21.2	10.7			1.35	13.1			
K. vulga					5.10			3.90	4.55	5.34	54.1	85.7	7143	3980	76.9	40.2	41.6
L. luna																	
Mytilina sp.					2.55				13.6	12.5	18.0	3.30	6.73			5.48	3.08
Platyias sp.					5.10	31.5	1840	3238	2627	626	7.22						
Polyarthra sp.	2276	478	92.5	44.9	2.55	360	1544	195	1.52								
Synchaeta sp.	56.4	95.4	299	19.3													

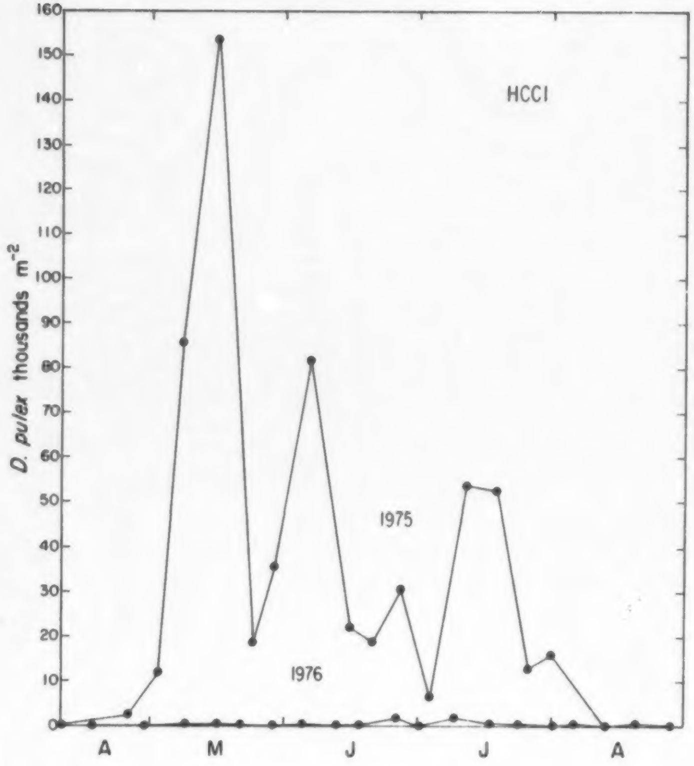

Fig. 68. *Daphnia pulex* densities in HCCl, 1975 & 1976.

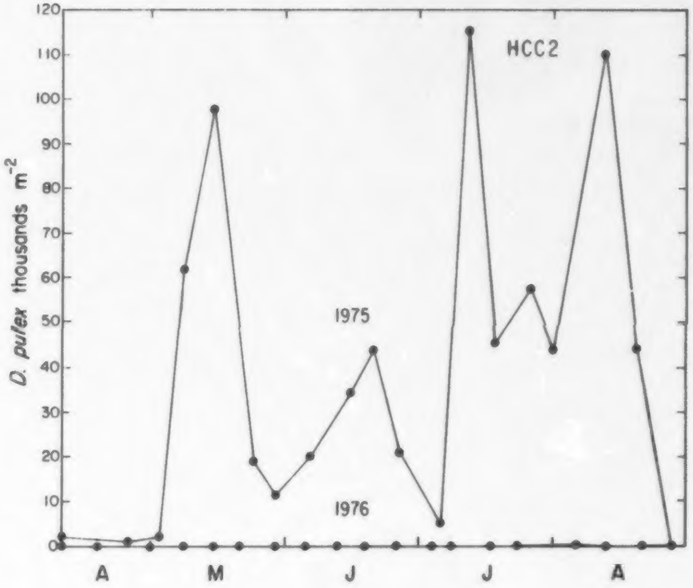

Fig. 69. *Daphnia pulex* densities in HCC2, 1975 & 1976.

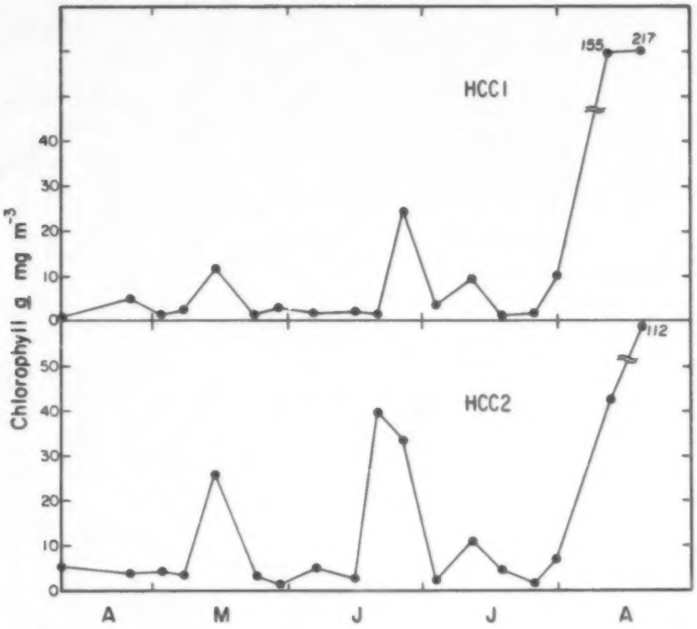

Fig. 70. Chlorophyll _a_ concentrations in HCC1 & HCC2, 1975.

Table 28: Chemical conditions and chlorophyll in HCC1 and HCC2, 1975. Chlorophyll was partitioned with a 44/um mesh. 1 = HCC1 2 = HCC2

		ICE														
1975		March				April		May					June			
		4	11	26	2	9	25	2	7	14	23	28	6	15	20	26
PO4-P	1	20	20	268	272	218	104	78	79	13	6	18	24	46	31	3
(ug/1)	1-bot			144	292	192	98	80	61	14	18	21	22	45	39	33
	2	29	20	246	216	196	80	75	61	10	20	50	32	43	4	9
	2-bot			298	214	264	65	77	62	10	27	58	32	47	45	44
Total Dis.-P	1	51	56	74	92	43	102	64	97	28	23	36	52	77	56	30
(ug/1)	1-bot			32	102	33	107	64	92	37	34	41	41	68	61	50
	2	82	59	54	48	30	86	63	97	30	38	65	55	64	30	40
	2-bot			56	55	53	66	69	91	30	42	74	59	109	46	65
Dis. Org. P	1	31	36				0		18	15	17	18	28	31	25	27
(ug/1)	1-bot						9		31	23	16	20	19	23	22	17
	2	53	39				6		36	20	18	15	23	21	26	31
	2-bot						1		29	20	15	16	27	62	1	21
Part. P	1	53	44	28	2	0	8	5	7	34	3	5	0	2	6	59
(ug/1)	1-bot			23	2	0	23	10	11	29	5	1	18	11	8	
	2	99	279	27	29	0	26	12	4	31	2	9	12	9	67	73
	2-bot			20	6	0	36	5	37	26	8	4	0	0	26	
Total P	1	104	100	102	94	38	110	69	104	62	26	41	52	79	62	89
(ug/1)	1-bot			55	104	33	130	74	103	66	39	42	59	79	69	
	2	181	238	81	77	29	112	75	101	61	40	74	67	73	97	113
	2-bot			76	61	49	102	74	128	56	50	78	59	91	72	
Chl a < 44um	1	0.5	0	0.4	0.3	1.0	5.0	1.5	2.1	11	1.2	2.8	1.7	1.3	1.0	1.4
(ug/1)	2	0.2	0	0.8	0.2	5.0	4.0	3.0	2.4	24	1.8	0.9	4.4	0.9	0.8	1.3
Chl a > 44um	1	0	0.1	0	0	0	0.1	0.1	0.3	1.0	0.5	0.2	0.3	1.0	0.4	23
(ug/1)	2	0.4	0.1	0.1	0	0.7	0.2	1.0	0.8	1.9	1.1	0	0.2	1.9	39	32
Tot. Chl a	1	0.5	0.1	0.4	0.3	1.0	5.1	1.6	2.4	12	1.7	3.0	2.0	2.3	1.4	24.4
(ug/1)	2	0.6	0.1	0.9	0.2	5.7	4.2	4.0	3.2	25.9	2.9	0.9	4.6	2.8	39.8	33.3

Table 28 Continued

1975		July				Aug.			
		3	11	18	25	1	12	19	27
PO4-P (ug/1)	1	5	18	30	50	32	5	10	12
	1-bot	35	37	62	47	43	10	9	12
	2	2	30	21	54	42	26	117	64
	2-bot	52	37	27	77	54	72	120	68
Total Dis.-P (ug/1)	1	51	57	73	100	47	41	43	60
	1-bot	72	78	107	84	69	37	41	60
	2	43	120	67	102	56	69	151	98
	2-bot	84	80	85	114	65	112	158	103
Dis. Org. P (ug/1)	1	46	39	43	50	15	36	33	48
	1-bot	37	41	45	37	26	27	32	48
	2	41	90	46	48	14	43	34	34
	2-bot	32	43	58	37	11	40	38	35
Part. P (ug/1)	1	10	36	13	14	9	61	177	72
	1-bot	15	20	15	21	23	59	115	79
	2	11	0	18	22	12	41	116	122
	2-bot	7	41	10	13	13	30	124	109
Total P (ug/1)	1	61	93	86	114	56	102	220	132
	1-bot	87	98	122	105	92	96	156	139
	2	54	104	85	124	68	110	267	220
	2-bot	91	121	95	127	78	142	282	212
Chl a <44um (ug/1)	1	2.6	1.7	1.1	1.7	5.4	14.2	21.7	
	2	1.9	1.1	0.8	0.5	1.9	2.6	3.7	
Chl a >44um (ug/1)	1	1.1	8.0	0.4	0.1	4.6	141	195	
	2	0.6	10	4.1	0.7	4.5	40	108	
Tot. Chl a (ug/1)	1	3.7	9.7	1.5	1.8	10.0	155	217	
	2	2.5	11.1	4.9	1.2	6.4	42.6	112	

Table 28 Continued

1975		March			April			May					June		
		ICE													
		4	11	26	2	9	25	2	7	14	23	28	6	15	20
NO_3 - N	1	592	816	390	342	395	360	335	385	315	240	220	188	155	138
+ NO_2 - N	1-bot			720	278	545	435	440	415	235	240	220	208	150	155
(ug/1)	2	448	550	330	288	390	425	380	425	300	280	230	196	175	120
	2-bot			270	308	390	315	410	400	245	280	220	196	155	165
NH_3 - N	1	210	84	895	550	680	420	300	293	16	86	84	190	173	156
(ug/1)	1-bot			1060	550	570	366	260	335	54	96	68	180	165	156
	2	206	132	840	540	590	366	340	312	30	146	120	84	118	17
	2-bot			765	520	480	423	330	335	30	160	216	90	130	156
pH	1	7.21		6.91	7.30	7.32	7.90	7.32	7.96	9.10	8.51	7.81	7.93	7.75	
	1-bot			6.91	7.10	7.25	7.43	7.23	7.68	9.03	9.01	7.72	7.83	7.77	
	2	7.03	7.43	6.86	7.20	7.20	7.40	7.30	7.69	9.20	7.89	7.61	8.05	7.61	
	2-bot			6.74	7.20	7.20	7.29	7.30	7.72	9.22	7.98	7.60	8.06	7.56	
Alkalinity	1			1.02	0.86	0.90	0.90	0.75	0.93	0.90	0.85	0.82	1.06	0.91	
(meq/1)	1-bot			1.64	0.63	1.14	0.86	0.69	0.78	0.82	0.86	0.78	1.00	0.90	
	2		1.49	0.59	0.69	0.79	0.67	0.70	0.82	0.82	0.83	0.67	0.90	0.76	
	2-bot			0.59	0.67	0.78	0.79	0.63	0.82	0.80	0.85	0.67	0.87	0.75	
Dis. Oxygen	1	2.85	1.86	9.89	9.01	8.00	7.90	9.50	9.40	12.35	7.38	7.10	6.61		
(mg/1)	1-bot	6.09	1.15	9.40		2.99	7.65	9.55	9.26	12.34	9.00	6.81	7.03		
	2	3.27	1.75	10.45	7.97	8.75	8.11	9.66	9.23	12.20	6.35	6.07	7.82		
	2-bot	4.40	1.82	1.35	7.06	6.56	5.17	9.60	9.10	12.25	5.70	5.65	7.82		
Secchi Disc,	1						.46	.91	1.55	1.52	$\underline{2.74}$	$\underline{2.90}$	$\underline{2.54}$		
(m)	2						.33	.86	1.52	1.24	1.52	$\underline{2.44}$	$\underline{1.96}$		
(__ = bottom)															

Table 28 Continued

1975		June	July				Aug.			
		26	3	11	18	25	1	12	19	27
NO_3 - N + NO_2 - N (ug/1)	1	16	60	188	19	24	19	8	13	24
	1-bot	6	49	45	13	18	18	2	5	21
	2	2	53	720	124	150	75	20	21	25
	2-bot	2	112	384	140	151	92	72	14	20
NH_3 - N (ug/1)	1	0	29	6	22	58	95	10	131	28
	1-bot	105	50	27	75	46	255	19	118	28
	2	1	12	28	31	65	163	35	187	33
	2-bot	1	70	41	38	82	173	216	42	45
pH	1	9.18	7.73	7.46	7.35	7.76	8.08	9.82	9.33	9.30
	1-bot	7.20	7.32	7.31	6.85	7.70	7.67	8.74	9.38	9.35
	2	9.80	7.72	7.50	8.15	8.03	8.20	8.62	7.95	8.82
	2-bot	7.50	7.15	7.40	7.90	7.97	7.97	7.87	8.07	8.50
Alkalinity (meq/1)	1		0.78	0.86	0.90		1.04	0.94	1.06	1.04
	1-bot		0.91	0.81	0.86		1.02	0.83	1.02	1.04
	2		0.71	0.75	0.96		1.31	1.14	1.39	0.81
	2-bot		0.45	0.78	0.90		1.35	1.20	1.37	0.80
Dis. Oxygen (mg/1)	1		6.45	7.00	6.49	5.80	7.06	18.45	9.27	
	1-bot						5.25	8.44	9.23	
	2		6.76	7.52	7.26	5.07	5.70	10.05	5.72	
	2-bot						5.30	5.55	6.11	
Secchi Disc, (m) (__ = bottom)	1	1.98	2.57	1.98	2.74	2.54	2.13	0.94	0.71	0.79
	2	1.68	2.59	1.80	2.26	2.29	1.98	1.85	0.89	0.63

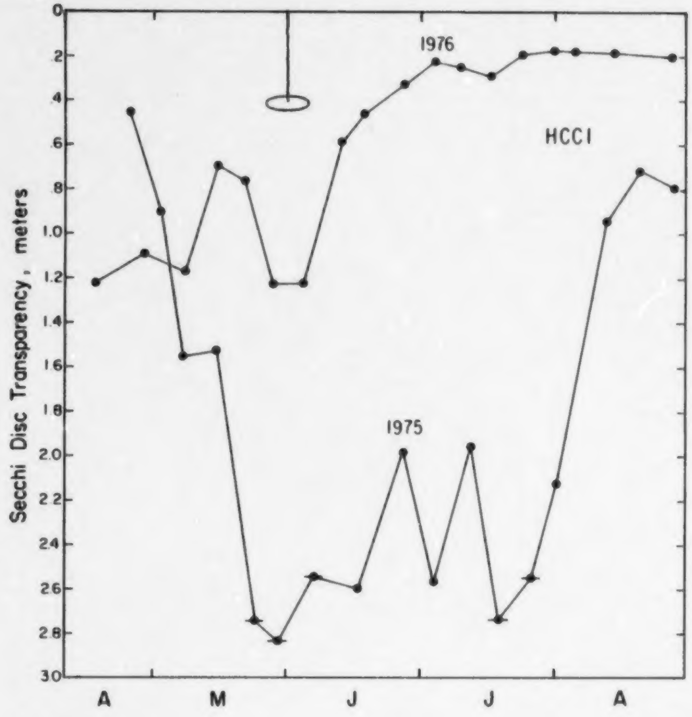

Fig. 71. Secchi disc transparency in HCC1, 1975 & 1976. (— = bottom.)

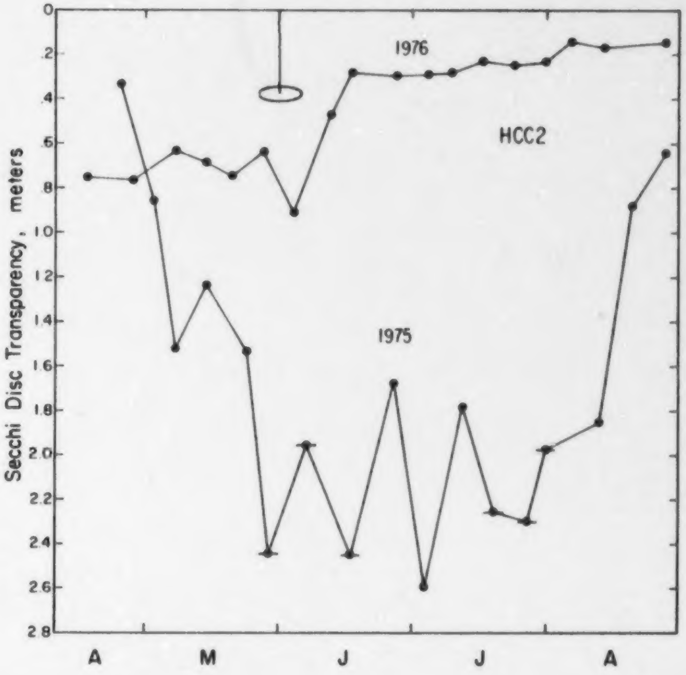

Fig. 72. Secchi disc transparency in HCC2, 1975 & 1976. (— = bottom.)

transparency measurements survive and show (Table 29) that transparencies were very much lower in 1976 than in 1975. Furthermore, although algae were abundant, Aphanizomenon flos-aquae did not form grass blades in 1976 in the absence of Daphnia pulex.

CONCLUSION

These experiments demonstrate the dramatic effectiveness of planktivorous fish in removal of large herbivores and the consequent dramatic effects on the phytoplankton. They also show that the system is complex, as witnessed by the fluctuations of the Aphanizomenon. The experiment on control of the planktivorous fish was terminated by the winter-kill, and so no conclusions can be drawn as to its ultimate success or failure.

ACKNOWLEDGEMENTS

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Table 29: Secchi disc transparency (m) HCC1 + HCC2, 1976.

Date, 1976	HCC1	HCC2
April 17	1.22	.76
28	1.09	.76
May 7	1.17	.63
14	.69	.69
20	.76	.74
27	1.22	.63
June 4	1.22	.91
12	.59	.46
17	.46	.28
26	.33	.30
July 3	.23	.30
9	.25	.28
16	.28	.23
23	.20	.25
30	.18	.23
Aug. 5	.18	.15
13	.18	.18
27	.20	.15

IV. B. THE EFFECTS OF FISH TOXICANTS ON PHYTOPLANKTON*

FILE DATA

One of the best ways to look at the roles of fish in lakes is to remove the fish and see what happens. As described in Section I, if benthivores were abundant, one of the effects might be to lower nutrient concentrations and, therefore, reduce the algal biomass. Another effect, if many planktivores were present initially, might be an increase in the numbers of larger herbivores which would lower the algal biomass. As many lakes in Minnesota have been "reclaimed" by the Department of Natural Resources, i.e. treated with fish toxicants, in the last 30 years, we examined existing records to see if any pattern would emerge. In all we found that 229 lakes had been treated between 1945 and 1976. Of these 138 were "cold water" lakes managed for trout, and 91 were "warm water" lakes managed for species such as bass, northern pike, and walleye. Because the effects we were looking for are unlikely in cold water lakes, we limited our search to the 90 warm water lakes. Furthermore, as little data other than fish information generally was collected we attempted to use Secchi disc transparency measurements where possible to determine whether the treatment had resulted in greater clarity of the water, presumably as a result of lessened fertility or increased herbivore abundance.

Sources for our data were as follows:

1. Minnesota Department of Natural Resources
   -- files of the Section of Fisheries
   -- files of the Section of Ecological Services
   --Carl's Lake progress reports.
2. Fremling, C., Lake Winona report, Winona State University 1973
3. Limnological Research Center, University of Minnesota,
   -- Transparency in Minnesota Lakes -- reports from the Secchi disc program.

Of the 91 lakes for which data was sought, 78 had to be eliminated because either pre or post-transparencies were unavailable or the lake was shallow enough so it winter-killed annually and was managed for water fowl rather than for fish. This left 13 lakes, for which the data are shown in Table 30. Using the data with care, i.e. giving little weight to Secchi disc values other than those for July through September, it appears as though seven of the lakes showed increases in transparency following treatment, two probably increased in transparency, and four probably showed no change. In some of the lakes the results were dramatic and long lasting. For example, transparency in Scandinavian Lake increased from about 1 m in August 1965, before treatment, to an average of 2.3 meters in August 1973 and 1975, and still averaged 1.4 meters in 1977. In Graham Lake transparency in early August before treatment was less than 0.3 meters. Three years after treatment transparency in early August was listed as greater than 1.5 meters.

In other lakes there were large increases that probably lasted for several years although the data do not exist to verify this. For example, in Fish Lake the average transparency for July and August before treatment was about 1.0 meter. In 1961, in the second post-treatment year, the July 6 value was 4.0 meters. Little Mud Lake on July 31, 1970 was listed as "turbid". On August 12, 1971, the year following treatment, the transparency was 4.4 meters.

Two of the lakes, Ham and Christina, showed no increases in the first years after treatment but did eventually become much more transparent. We know of no reasons for the increase other than the treatment. It may be that those lakes in which planktivorous fish were abundant responded more quickly, because of the short generation time of the herbivorous zooplankters, than those lakes in which the effect was caused by the elimination of bottom-feeding fish. In the latter elimination of the nutrients by the fish would not be felt immediately, but the lake response would be a gradual lessening of fertility.

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*by Joseph Shapiro and Eric Smeltzer

Clear Lake is an interesting example where the lake did respond to toxaphene, but unfortunately the year after treatment the lake was restocked with bottom feeders and planktivores and so it did not remain transparent.

In addition to the 12 lakes listed, Minnesota DNR personnel can list a number of lakes that they recall as having responded to treatment with increased transparency, as well as some lakes that did not respond in this way. However, data are not available to document these observations.

In balance, then, it would appear that use of fish toxicants does reduce algal abundance and that in some cases the effect has been longlasting.

Table 30: The effect of fish toxicants on lake transparency: data from the files of the Minnesota Department of Natural Resources.

Lake, County, Date of Treatment, Toxicant	Date	Secchi Disc Transparency (ft)	Comments
Scandinavian,	6-23-55	4.0	large increase in transparency lasting at least 8 years
Pope	8-65	3.3	
11-6-67	7-69	15.5	
Toxaphene	8-28-73	7.5	
	5-28-74	13.0	
	8-28-75	7.5	
	1977 mean	4.5	
Fish,	7-16-47	2.9	large increase in transparency lasting at least 2 years
Jackson	8-15-50	4.1	
11-4-59	8-20-56	2.7	
Toxaphene	7-6-61	13.0	
Carls,	6-23-58	2.0	transparency increase after winterkill 1974-5 and after rotenone treatment 1975
Scott	1974 mean	1.9	
9-22-75	1975 mean	4.1	
Rotenone	1976 mean	6.3	
Little Mud,	7-31-70	"turbid"	transparency increased
Meeker	5-29-71	11.5	
9-15-70	6-19-71	13.8	
Rotenone	8-12-71	14.5	
Graham,	8-2-54	<1.0	transparency increased greatly, possibly lasting for at least 3 years
Carlton	8-7-58	>5.0	
9-23-55			
Toxaphene			
Clear,	7-22-51	2.5	transparency increased slightly
Waseca	6-24-57	2.5	
10-25-63	9-19-60	1.5	
Toxaphene	6-11-63	1.3	
	8-12-63	1.3	
	1963 mean	1.8	
	6-16-64	5.0	
	8-4-64	3.1	
Bear,	7-23-57	2.0	transparency increased slightly
Freeborn	8-59	>3.5	
11-13-58	6-24-61	1.2	
Toxaphene			

Table 30 Continued

Lake, County, Date of Treatment, Toxicant	Date	Secchi Disc Transparency (ft)	Comments
Winona, Lower Lake Winona 9-17-73 Rotenone	7-21-68	2.5	high transparency in early summer 1974, but transparency declined to pre-treatment levels in the summer
	10-10-72	2.0	
	9-17-73	1.7	
	10-15-73	3.5	
	5-8-74	14.8	
	5-15-74	4.8	
	6-17-74	8.5	
	6-20-74	9.1	
	6-26-74	6.0	
	8-8-74	3.0	
	9-17-74	1.8	
Bud, Martin 9-10-67 Rotenone	7-31-47	2.7	high transparency in early summer 1968, but declined to pre-treatment levels late in summer
	7-31-54	2.0	
	4-19-67	1.5	
	5-22-67	2.8	
	6-20-67	2.8	
	7-18-67	10.5	
	8-23-67	5.0	
	5-15-68	10.0	
	6-12-68	>13.0	
	7-16-68	3.4	
	6-5-70	11.5	
	1975 mean	3.0	
Christina, Douglas 11-4-65 Toxaphene	?-47	5.0	possible delayed transparency increase
	?-50	3.0	
	?-52	6.0	
	?-56	4.0	
	?-59	0.7	
	?-61	0.5	
	?-63	0.8	
	8-22-67	1.0	
	?-68	1.6	
	8-12-72	6.5	
	9-8-76	3.0	
Ham, Anoka 10-18-55 Rotenone	8-24-26	2.0	possible delayed transparency increase
	9-21-48	1.0	
	8-22-56	1.9	
	8-1-74	3.7	
	1975 mean	7.0	
	1976 mean	6.0	

Table 30 Continued

Lake, County, Date of Treatment Toxicant	Date	Secchi Disc Transparency (ft)	Comments
Anka,	1947 range	8 - 10.5	no transparency increase
Douglas	8-18-50	1.5	
11-4-65	8-22-67	1.7	
Toxaphene	?-68	1.7	
	?-72	4.5	
Winona, Upper Lake	7-21-68	2.3	no transparency increase
Winona	10-10-72	1.8	
9-17-73	9-17-73	1.3	
Rotenone	5-15-74	4.0	
	6-6-74	1.9	
	6-13-74	2.8	
	6-20-74	2.5	
	6-26-74	2.1	
	7-8-74	1.5	
	10-7-75	12.7	

EFFECTS OF ROTENONE IN WIRTH LAKE*

One lake in which we have been able to study the effects of rotenone treatment in detail is Wirth lake in Minneapolis. This was not our original intent, which was to study the effects of added piscivores. The situation became complicated, but the results are of interest.

Wirth Lake (area 16 ha, mean depth 4.3 m) is managed by the Minneapolis Park and Recreation Board as a swimming and fishing facility. Because of its location it has long received considerable amounts of urban runoff as well as periodic inputs of nutrient-laden Bassett Creek water during floods. In consequence it is eutrophic with the characteristic features of low transparency, large populations of algae dominated by blue-greens, anoxic hypolimnion, etc. Our first study of the lake, where these factors were described, was done in 1974 as part of a survey of lakes controlled by the Minneapolis Park Board (Shapiro 1974). In 1974, upon learning that the Minnesota Department of Natural Resources planned to remove all adult northern pike (_Esox lucius_) from Lake Harriet, another Park Board lake, in Spring 1975, we asked them to stock a portion of the pike into Wirth Lake. This was to determine whether these predators could lessen the abundance of planktivorous fish in the lake, thereby relieving the larger herbivorous zooplankters in turn from predation. If this were to occur, the resulting increase in herbivore activity could materially lower algal abundance, thereby improving the lake for the recreational activities. Accordingly, plans were made, and in April, 1975, 212 adult northern pike were stocked into Wirth lake. The intent was to continue to monitor the appropriate aspects of the limnology of the lake to evaluate the efficacy of the procedure. This plan was carried out through 1976, but in 1977 several things were done to Wirth Lake that made it difficult to follow the original plan: In August 1977 the Park Board, wishing to alleviate the eutrophic state of the lake, hired an engineering consultant who built a peat bed beside the lake and began to pump hypolimnetic lake water through it in an attempt to remove phosphorus from the water. Unfortunately, at the same time, the consultant installed eight aerators on the lake bottom and put them into operation. Because of this, hypolimnetic phosphorus, which normally would not enter the euphotic zone, became diluted throughout the lake. Furthermore in September, 1977, the DNR after removing some of the fish from the lake treated the lake with rotenone. Restocking began in October 1977 and continued into 1978. Examination of stocking records also reveals that stocking had begun in 1974 and continued after 1975. Thus a great deal has happened to the lake. However, we have continued to follow phenomena in the lake in an effort to evaluate the effects of the manipulations. A summary of the events is presented below:

A. Studies by Limnological Research Center
   1974 -- June-September, temp, O2, transparency, Chl, N, P, algae, zooplankton, echosounding
   1975 -- June-September, same as above
   1976 -- May-August, same as above
   1977 -- June-November, same as above
   1978 -- May-September, same as above
B. Fish stocked by Minnesota DNR
   1974 -- 150 northern pike -- yearlings
   1975 Spring -- 212 northern pike adults
   1976 January -- 115 northern pike -- yearlings
   1976 March -- 52 northern pike -- adults

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*by Joseph Shapiro

C. Fish removed by net by Minnesota DNR
    1977 -- August-September, 5500 crappies, 4200 sunfish, 14 northern pike

D. Rotenone treatment by Minnesota DNR
    1977 -- September 13

E. Fish restocked by Minnesota DNR
    1977 -- October, 947 walleye fingerlings, 1050 large mouth bass,
            fingerlings, 2390 Channel catfish
    1978 -- Spring, carp, adult (by fishermen)
    1978 -- May, 11,200 bluegill, adults
    1978 -- June, 9600 largemouth bass, fry, 15,000 walleye, fry

F. Lake level change

In 1977 a barricade was built to prevent Bassett Creek from overflowing into the lake and to isolate the fish population. The lake level was thus raised 1.2 m and its area increased.

G. Artificial circulation and aeration
    1977 -- August, circulators first turned on.
    1977-78 -- Winter, circulation done to prevent winter-kill by low oxygen under ice resulting from flooding of land area by level increase.

H. Phosphorus removal
    1977 -- August, water was pumped through the peat bed but measurements by the Minnesota Pollution Control Agency indicated little, if any, removal of phosphorus.

Phytoplankton

Because the purpose of the original stocking with northern pike in 1975 had been to reduce the algal abundance, it is appropriate to look first at what did happen to the algae.

Chlorophyll

Surface chlorophyll concentrations for 1974-1978 are shown in Table 31 and plotted in Figure 73. Chlorophyll generally was at moderate levels of 10-25 mg m-3 in Spring and rose during July, August and September to as high as 69 mg/m-3 in 1974 and to 85 mg m-3 in 1978. The increase in 1978 may have been occasioned to a degree by phosphorus entering the epilimnion from the hypolimnion. However, generally, total P in the epilimnion remained constant through mid-August (Table 31, Fig. 74) and stratification was stable until about the same time. Thus there was a progressive increase in the chlorophyll/P ratio (Table 31). As the ratio increased from about 0.2-0.5 towards 1.0, either some other substance, such as nitrogen, was becoming available by runoff or fixation, and making the use of P more efficient, or some agent resulting in reduction of chlorophyll was becoming less effective. Fixation is possible, as species capable of it were present, and in 1974-1975 when nitrate levels were measured they were very low, although during the same years ammonia was present in the surface waters in significant amounts. A chlorophyll-reducing agent may be found in the population of herbivorous zooplankters and, as seen in Table 32 there is, in recent years, including 1978, a tendency for cladocerans to decline from Spring to mid-August. In any event, in 1975 and 1978 the pattern was different. In Spring 1978 the chlorophyll levels were very low, and in both years chlorophyll concentrations fell precipitously in late summer.

Algal Species

No determinations were made for 1975, but in 1974, 1976, and 1977, blue-greens were predominant. In 1974, Aphanizomenon flos-aquae and various species of Anabaena were dominant until September, when Oscillatoria became abundant. In 1976, *Aphanizomenon* gave way to a mixture, *Aphanizomenon* and *Anabaenopsis*, then of *Aphanizomenon*, *Anabaenopsis* and *Oscillatoria*. In June-August, 1977, *Oscillatoria* was abundant, followed by *Aphanizomenon* and *Anabaena*. However, in 1978 the picture was different. Through early July blue-greens were very scarce, with most of the few algae present being greens -- cryptomonads or *Schroederia*. By July 20 however, flakes of *Aphanizomenon flos-aquae* dominated the now-abundant algae, and continued to do so along with some *Ceratium* and *Microcystis* until August 20 when the flakes disappeared, reappearing again as smaller flakes on August 30.

Transparency

The changes in algal abundance, and to an extent in species composition, manifested themselves in changes in Secchi disc transparency, as shown in Table 31 and Figure 75. In 1974, 1975 and 1977, summer transparencies were less than a meter and as low as 0.6 m. However 1976, and especially 1978, transparencies were very much greater. In fact, in 1978, Secchi disc transparency exceeded 2 m into August and was as high as 4.6 m on July 4. One apparent result was an increase in macrophyte abundance with reports of floating masses of weeds.

Total phosphorus

Surface total P concentrations are shown in Table 31 and Figure 74. Until August 1977, concentrations seemed relatively stable at 40-60 mg m-3. As 1975 had more rainfall than either 1974 or 1976, its higher values probably reflect the greater amount of urban runoff. However there is no doubt that the very high values in August-September 1977, and from May onward in 1978, were brought about by the air-induced circulation. Not only did they occur after circulation began, but Wirth Lake has exceptionally high concentrations of P in its hypolimnion. Figure 76 shows a plot of total P on September 15, 1975. In fact at one time because of the abundance of P in the hypolimnion, we considered using an Olzewski tube (Olzewski 1959) to drain P from the hypolimnion and export it to Bassett Creek.

Dissolved oxygen

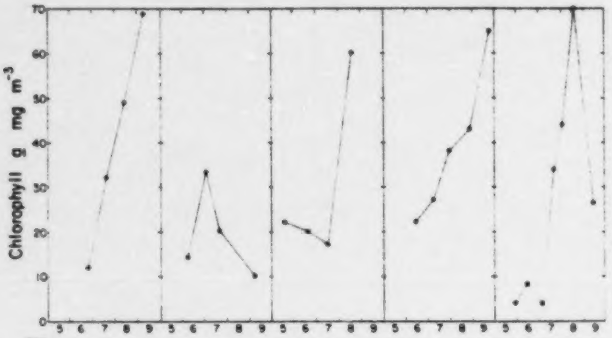
Dissolved oxygen data are shown in Figure 77, where vertical profiles for mid-August are drawn for the three years when the data were taken. Clearly, operation of the aeration devices resulted in the deeper waters becoming aerobic, and in a considerably greater fraction of the lake sediments being exposed to oxygenated water in 1977 and 1978 than in 1974-1976.

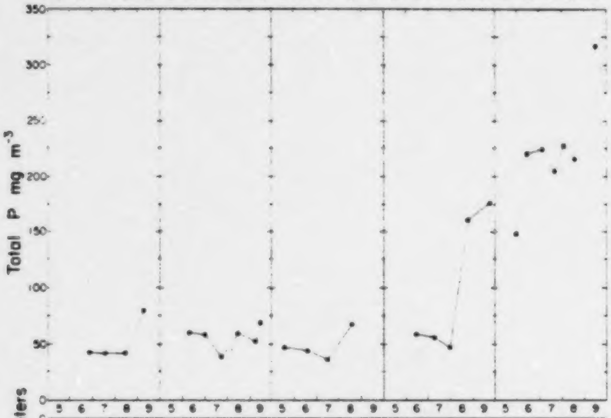
Zooplankton

Examination of Table 32 shows that Wirth Lake has a varied zooplankton population. A total of 11 cladocerans have been found, of which 6 are species of *Daphnia*. In addition, 4 copepods and at least 11 rotifers make up the community. Because of their role in controlling the algae, the larger *Daphnia* are of particular interest. Figure 78 shows the abundance of *Daphnia galeata* and *Daphnia pulex* from 1974-1978. *Daphnia galeata* was present in all five years, generally most abundant in Spring, reached its highest population of 486,000 m-2 in May 1976 and its lowest -- undetectable -- in August, 1978. Mean body length in this species has been as follows:

Table 31: Transparency, phosphorus and chlorophyll in Wirth Lake, 1974-1978.

	Secchi Disc, m	Total P mg m-3	Chl a mg m-3	Chl/P
1974				
June 26	1.13	43	12.3	.29
July 16	.64	42	31.5	.75
August 14	.61	42	49.4	1.18
September 10	.67	80	68.7	.86
1975				
June 9	1.22	60	14.0	.23
July 1	.70	58	33.0	.57
July 23	.79	38	20.0	.53
August 15	.76	59		
September 9	1.07	52	10.0	.19
September 15		68		
1976				
May 18	3.14	46	22.4	.49
June 18	1.52	44	19.9	.45
July 15	1.52	36	16.7	.46
August 17	.61	67	59.1	.88
1977				
June 14	.76	58	22.0	.38
July 7	.70	55	27.0	.49
August 1	1.01	47	38.0	.81
August 24	.85	160	43.0	.27
September 23	.88	176	65.0	.37
November 8	.91	182	80.0	.96
1978				
May 29	3.66	148	4.1	.03
June 14	3.51	220	8.3	.04
July 4	4.57	224	3.6	.02
July 20	2.13	204	33.8	.17
August 2	2.29	227	43.6	.19
August 18	1.37	215	85.0	.40
September 15	1.46	317	26.0	.08





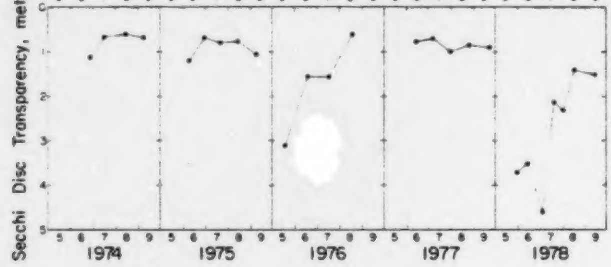


Fig. 73. (Top) Chlorophyll a concentrations in Wirth L., 1974 - 1978.

Fig. 74. (Center) Total phosphorus concentrations in Wirth L., 1974 - 1978.

Fig. 75. (Bottom) Secchi disc transparencies in Wirth L., 1974 - 1978.

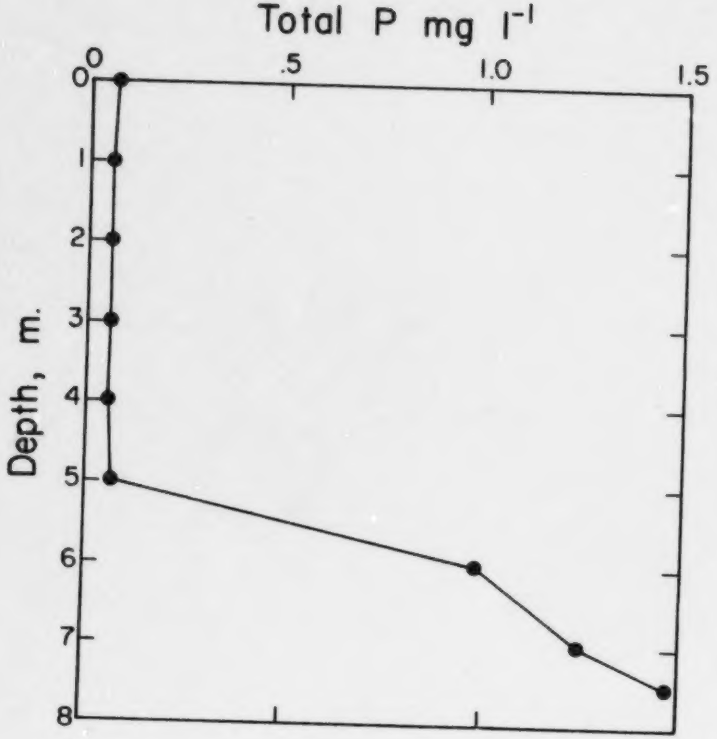


Fig. 76. Total phosphorus profile in Wirth L., Sept. 15, 1975.

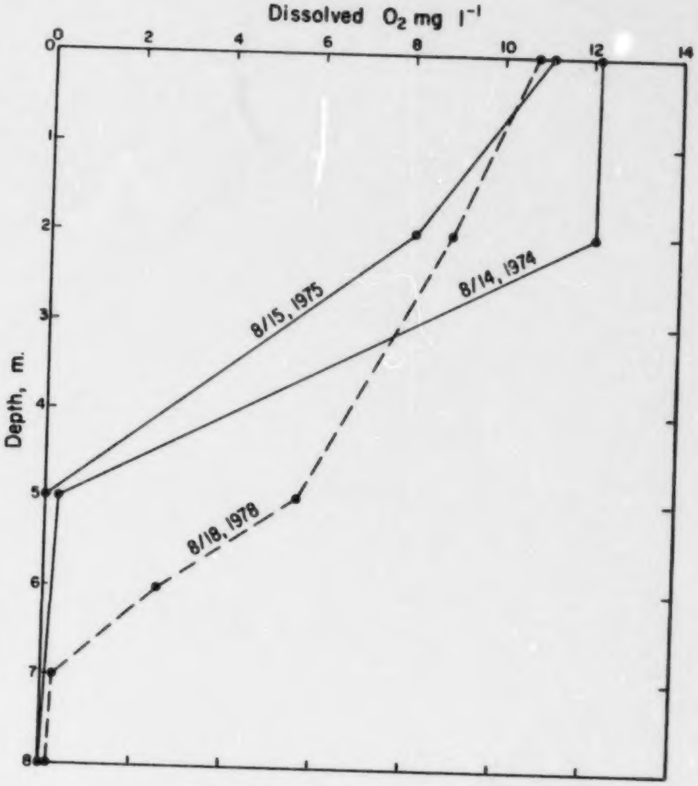


Fig. 77. Dissolved oxygen profiles in Wirth L. before (1974 & 1975) and after (1978) aeration.

Table 32: Zooplankton (thousands of individuals m^{-2}) in Wirth Lake, 1974-1978.

	1974				1975					1976			
	June 26	July 16	Aug 14	Sept 10	June 9	July 1	23	Aug. 15	Sept 9	May 18	June 18	July 15	Aug 17
CLADOCERA:													
Daphnia pulex					13	9	2						
Daphnia galeata	27	7	24	152	125	169	93	26	46	486	108	88	58
Daphnia retrocurva			4		4					7	1	8	8
Daphnia longiremis					15					183		3	
Daphnia parvula					4							3	
Daphnia ambigua										65			
*Bosmina longirostris	151	3	110	359	275	594	5	362	307	131	4		175
Ceriodaphnia sp.	25	209	420	182	2	2	4	2	32	46			60
*Chydorus sphaericus	47	91	20	43		6	40	7	52		6	23	142
Diaphanosoma leuctenbergianum	3	10	157	113		4	44	155	115		6	248	258
*Leptodora kindtii													
Total Cladocera less *	55	226	605	447	163	184	143	183	193	787	115	350	384
COPEPODA:													
Cyclops bicuspidatus	30			17	218	4			9	135	6	2	22
Cyclops vernalis		7			5		4	4	14				29
Mesocyclops edax	130	33	43	107	32	165	84	57	83	32	117	110	315
Diaptomus siciloides	66		55	36	42	86	45	81	129	23	24	84	39
Nauplii (copepodids)	3	333	177	197	51	117	58	44	195	303	223	488	416
ROTIFERA:													
Asplanchna		23			51	15				3			
Conochilus		688		48		1506							
Filinia		20						2				1	143
Kellicottia longispina	2	3		54						166	42	3	2
Keratella cochlearis		20	4	3	35	6		2	3	8015	45	191	601
Keratella quadrata	2				9					42	16		
Polyarthra vulgaris		3	24	5						3	35	85	195
Trichocerca multicrinis		7				2							
Trichocera similis													12
Platyias patulus													
Brachionus sp.													

Table 32 Continued

	1977						1978						
	June 14	July 7	Aug 1	24	Sept 23	Nov 8	May 29	June 14	July 4	20	Aug 2	18	Sept 15
CLADOCERA:													
Daphnia pulex							145	441	182	471	233	209	
Daphnia galeata	51	33	35	79		56	143	12	9	17	6		
Daphnia retrocurva	24	50	142	130						3			
Daphnia longiremis													
Daphnia parvula			3										
Daphnia ambigua	9		3										
*Bosmina longirostris	21		32	66		6	2	2	2	20	9	5	103
Ceriodaphnia sp.	539	97	61	9									
*Chydorus spaericus			19	158				2		12	9	32	53
Diaphanosoma leuctenbergianum	42	50	142	149						3	1	11	142
*Leptodora kindtii			3										5
Total Cladocera less *	665	230	386	367	0	56	288	453	191	494	240	220	142
COPEPODA:													
Cyclops bicuspidatus			3	28	12	1575	83			3			
Cyclops vernalis													
Mesocyclops edax	30	52	176	222	2	3		45	52	64	10	5	11
Diaptomus siciloides	88	78	43	98				17	56	84	53	27	319
Nauplii (copepodids)	312	240	473	436		97	143	143	871	1294	329	268	500
ROTIFERA:													
Asplanchna			5	6									5237
Conochilus												11	5
Filinia	185	50	16	3									66
Kellicottia longispina		105	104	949									
Keratella cochlearis	1132	190	524	468	2		5	2				11	
Keratella quadrata	15	10					88	7					566
Polyarthra vulgaris	9	10	3	247						3	6	510	36
Trichocerca multicrinis			37										
Trichocera similis		14		28									58
Platyias patulus										3			
Brachionus sp.										145			

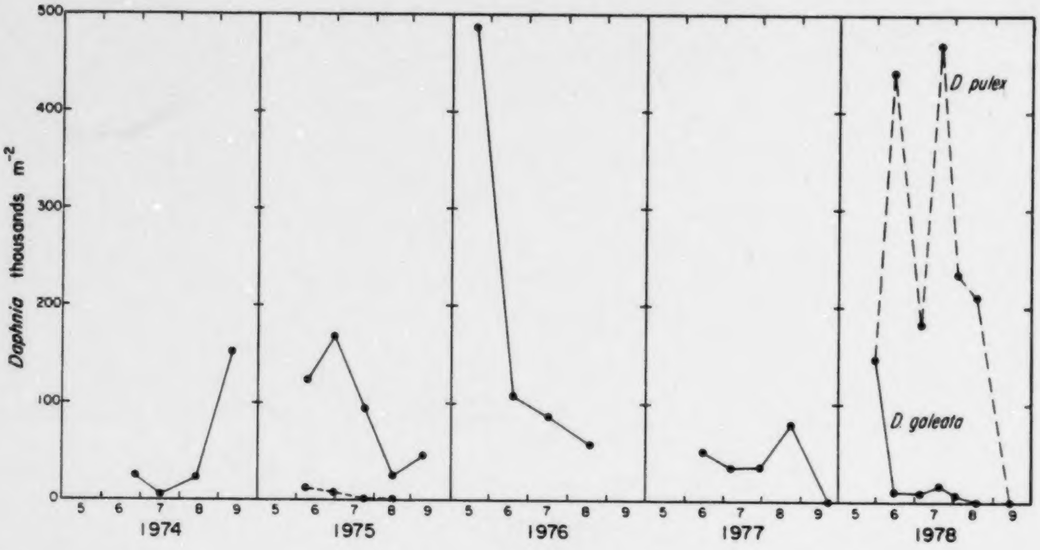


Fig. 78. Abundance of Daphnia pulex and Daphnia galeata in Wirth L., 1974 - 1978. (Rotenone treatment was done on Sept. 13, 1977.)

Year	Mean Body Length, mm
1974	.883 (July and August only)
1975	.992 (June, July and August)
1976	.878 (June, July and August)
1977	.791 (June, July and August)
1978	1.00 (May only)

Too few specimens were present in 1978 to determine body lengths for other than the May sample. Daphnia pulex was found in only two of the five years -- 1975 when it occurred at a maximum of 13,000 m-2 and was gone by mid-August, and 1978, when it occurred at up to 471,000 m-2 disappearing probably in late August. Body lengths were considerably greater than those for Daphnia galeata, as follows:

Date	1978 Mean Body Length, mm
5/39	1.39
6/14	1.44
7/4	1.70
7/10	1.38
8/7	1.46
8/18	1.32

Ten days following rotenone treatment on September 13, 1977, no Daphnia were found -- indeed the only zooplankters recorded were a few Cyclops, Mesocyclops, and Keratella. However, by November 8, 1977, Daphnia galeata was found in respectable numbers and Bosmina and Diaptomus had recovered as well. Cyclops bicuspidatus was present in very great abundance, possibly because of the abundance of food and absence of competitors and predators.

Several species seem to show a distribution inverse to that of D. pulex. For example, in 1978, Bosmina and Diaphanosoma were rare until D. pulex disappeared. Similarly, Ceriodaphnia was abundant except for 1975 when it was sparse, and 1978 when it was absent. Kellicotia was also present except for the two years when D. pulex appeared, and Chydorus sphaericus was rare in the early part of 1978. Even. D. galeata was in least abundance in 1978 when D. pulex was abundant.

DISCUSSION

Wirth Lake has undergone a variety of manipulations and has exhibited a number of changes. The questions are, what are the causal relationships, and what can be learned? The most obvious change in the lake was the exceedingly small algal population during early 1978, which resulted in the very great transparencies during the same period. Two possible explanations present themselves -- either the growth rate of the algae was low, or the loss rate was high. It is unlikely that the growth rate was low. Rainfall and therefore runoff to the lake during early 1978 were above average and, in addition, operation of the circulators increased the nutrient concentrations. Furthermore, it is not likely that circulation caused light limitation as transparency was very high, and the algae increased greatly during July and August even though circulation continued and transparency declined. The most likely explanation lies in the grazing activities of the very large population of D. pulex, which averaged 256,000 m-2 or, considering the depth of the zooplankton tow (8 m) and the completely mixed water column, 32 individuals per liter. This concentration is within the range of that capable of removing from the water essentially all filamentous blue-greens (Lynch and

Shapiro 1980, see Section IV.A.). In fact, very few blue-greens were present during the early part of 1978, being replaced by green algae that must have been growing very rapidly to be able to maintain themselves in the face of such intense grazing. This is similar to what happened in Severson Lake, Minnesota, as a result of a large population of D. pulex (Schindler and Comita 1972). Low phytoplankton standing crops with no blue-greens but with diatoms and green flagellates were reported.

Two of the manipulations may have contributed to the large population of D. pulex in 1978 -- the circulation and the rotenone treatment. The circulation may have helped because, as shown by echosoundings taken in Wirth Lake in June 1977 and as expected from the dissolved oxygen distribution, the fish population had been confined to the epilimnion. Also, because of the anaerobic conditions in the hypolimnion, the Daphnia, being without a refuge would also be in the epilimnion and subject to predation. Circulation, because of dilution of both populations, would be expected to reduce this predation and result in an increase in the Daphnia population as it did in Lake Calhoun during circulation there (Shapiro and Pfannkuch 1973). Had sampling been done in September 1977, after circulation began, but before the rotenone treatment, any such increase in Daphnia could have been detected. Unfortunately, this was not done and so the question is moot. On the other hand, there is little doubt that the elimination of the fish (of which 50% were crappies, 25% bluegill sunfish, 15% carp, and 10% perch, bullhead, sucker, northern pike, and largemouth bass) by the rotenone treatment was largely responsible for allowing D. pulex to exist and become abundant in the lake in 1978. The appearance of this species is common following reduction of predation on it because of either winter-kill (Schindler and Comita 1972; Shapiro, Section IVC) or circulation (Shapiro, and Pfannkuch 1973 -- Lake Calhoun); as is its rapid disappearance following the addition of predators (Lynch 1979). What makes D. pulex so sensitive is its size and therefore its visibility to the fish. For example the D. pulex in Wirth Lake in 1975 averaged 1.45 mm body length -- compared with the average of 0.89 mm for the D. galeata in the lake from 1974-1977. On May 29, 1978 D. galeata were larger than usual, averaging 1.00 mm long -- higher than on May 18, 1976 (0.86 mm), or on June 14, 1977 (0.82 mm). One interesting feature is that D. galeata did not reach very great abundance in November 1977, following its recovery from rotenone treatment, even though it seemingly had few competitors. Furthermore, D. galeata has existed in the lake continuously in the face of fish predation. It thus seems as though this species, although it may respond somewhat by increasing its size in the absence of predation is, by contrast with D. pulex, controlled by factors other than predation, at least under normal predator levels.

The original intent in studying Wirth Lake was, as noted, to evaluate the effect of adding carnivorous fish. Although the later manipulations did result in more dramatic changes, there is evidence that the stocking with northern pike may have had some effect. Northern pike had been added in 1974, 1975 and 1976. These stockings, particularly of adults, may have been efficacious in reducing the abundance of one or more planktivores. In July 1975, northern pike in Wirth Lake were rated by the DNR as being few in number, by September 1975 they were near the state average and by August 1977 they were well above the average. At the same time, perch which were abundant in September 1975 were, by August 1977, reduced in numbers. One might have expected such a progression to lead eventually to the appearance of D. pulex in the lake, assuming the perch were their chief predator. The rotenone treatment made it impossible to tell. Strangely enough, D. pulex did appear -- but in 1975 -- suggesting a great decline in planktivore abundance or activity in that year. Further evidence of such a decline lies in the fact that *D. galeata* in 1975 averaged .99 mm in length, compared with .85 m in 1974, 1976, and 1977. Chlorophyll *a* also was different in 1975, showing an uncharacteristic decline (Fig. 73). To what extent these phenomena are related to the stocking of northern pike is unclear. It is possible that the pike stocked in 1974 and 1975 were the cause for the *D. pulex* increase in 1975, but the uncharacteristic decline in chlorophyll came about during the period when *D. pulex* was rare or absent.

The other anomalous relationship occurred in summer 1978. Until July 4, and probably for some time afterwards, blue-green, and in fact all algae, were scarce, probably because of the grazing pressure of the large population of *D. pulex*. However, on July 20 chlorophyll levels were high and remained so until the end of sampling on September 15. The explanation lies in the ability of *Aphanizomenon* to maintain so-called "grass-blade" blooms in the presence of large *Daphnia*, such as *D. pulex*. Lynch (1980) believes that once *Aphanizomenon* colonies reach a size large enough to escape predation by *Daphnia*, they prosper as the *Daphnia* remove algal competitors and release nutrients from them. In order for the colonies to become large enough to escape predation in the first place the algae must be able to grow where they will not be grazed. Lynch believes that this may occur at the sediment surface where new colonies may form from akinetes. This idea is strengthened by the failure of *Aphanizomenon* to form such large colonies in enclosures, where the water is isolated from the sediments, or in places where the hypolimnion is anoxic. In other words, two circumstances favor the production of large ungrazable *Aphanizomenon* colonies -- many large *Daphnia* -- such as *D. pulex*, and an oxic hypolimnion. Both were present in Wirth Lake in 1978, the first as a result of the rotenone, the second because of artificial circulation. It is possible that had artificial circulation not been done, the *Daphnia* would have been able to control the algae beyond mid-July. Interestingly, on September 15, 1978, when no *Daphnia* were present, the grass-blade colonies had disappeared, and the transparency which had been high despite the abundant chlorophyll present in the large algal colonies, increased only slightly even though the chlorophyll declined to less than a third of that on August 15.

CONCLUSIONS

The conclusion from this study is that, although Wirth Lake was beginning to respond to the stocking of northern pike, in that the number of perch declined, the greatest influence on the lake resulted from the rotenone treatment. By eliminating planktivorous fish *D. pulex* was allowed to increase and began to control the algal population to the point where transparency increased greatly despite the large increase in phosphorus concentrations resulting from circulation. The circulation had two detrimental effects: it made it more difficult for the peat bed to remove any significant fraction of the phosphorus in the lake and it allowed *Aphanizomenon* to form grass blade blooms.

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THE INFLUENCE OF FISH ON THE ABUNDANCE OF ALGAE IN CLEAR LAKE, MINNESOTA*

It has been observed that lakes often respond to fish-kills with reduced phytoplankton abundance the following year. To study the ecological mechanisms underlying this phenomenon, a whole-lake experiment was designed involving Clear Lake, Minnesota. The purpose of the study was to assess the relative importance of two possible mechanisms linking fish-kills to algal declines:

1. Removal of planktivorous fish results in increased zooplankton abundance and/or increased dominance by large-bodied zooplankton species. Algal abundance declines in response to increased grazing pressure.
2. Removal of benthivorous fish eliminates a source of nutrient regeneration from the sediments to the water. Algal abundance declines in response to a reduced supply of nutrients.

Clear Lake (21 ha, 2 m max. depth) is divided into two basins, North (N) and South (S), by a road and causeway. Clear S, in 1978, contained populations of benthivorous black bullheads (Ictalurus melas) and planktivorous fathead minnows (Pimephales promelas). Clear N, in 1978, was void of fish as a result of a previous winter-kill. Both basins were treated with the fish toxicant rotenone in Fall, 1978, so that fish were virtually absent in 1979. Thus, the natural fish-kill during winter in Clear N and the artificial fish-kill by rotenone created a controlled experimental situation in which the response of the two basins to fish removal could be studied.

The removal of fish from Clear Lake both by winter-kill and by rotenone treatment resulted in sharp declines in algal abundance. Increased grazing of algae by zooplankton in the absence of fish was an important mechanism leading to the algal decline. This conclusion was supported by the following observations.

1. The zooplankton community was dominated by large herbivores in the fish-kill situations.
2. Algal losses to non-respiratory causes increased in importance in the production and loss dynamics of the algal community after fish-kill.
3. Non-respiratory loss rates were correlated with herbivorous zooplankton biomass.
4. The population of Daphnia pulex present in Clear S after fish-kill was physically capable of filtering food particles at a rate sufficient to account for the observed non-respiratory losses suffered by the algae.
5. The specific rate of photosynthesis increased after fish-kill.
6. There was a shift in the nutrient pools in favor of the dissolved fractions after fish-kill.

There was, however, some evidence against the importance of grazing in the fish-kill situations.

1. No inverse correlations were observed (within a season) between algal and zooplankton abundance.
2. Bag experiments failed to demonstrate a positive growth response by algae to release from grazing.

Reduced levels of TP and TN were observed in the absence of fish. The decline in nutrient levels may have accounted for about half of the extent of the algal decline in Clear S following fish-kill. However, the elimination of nutrient regeneration from benthivorous fish excretion could not have caused the observed nutrient decline. This conclusion is supported by the following evidence.

1. Changes in TP levels during 1978 were synchronous between North and South basins, and were correlated with rainfall.

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*by Eric Smeltzer - thesis abstract

2. The nutrient decline following rotenone treatment occurred in Clear N, the control basin, as well as in Clear S.

3. The quantity of phosphorus excreted by bullheads to Clear S during 1978 was a very small fraction of the total annual load of phosphorus to that basin.

4. The decline in the quantity of phosphorus present in Clear S between 1978 and 1979 could not be accounted for by the loss of fish excretion as a phosphorus source.

The possibility remains that fish excretion could be an important mechanism linking fish kills to reduced algal abundance in lakes with more substantial benthivorous fish populations.

IV.C. EFFECTS OF WINTER-KILL ON MINNESOTA LAKES IN 1978-1979*

Because the winter of 1978-1979 was particularly long, with snow covering the ice for most of the period, a considerable number of Minnesota Lakes suffered winter-kill. Therefore we took the opportunity to study a number of these lakes along with what appeared to be control non winter-kill lakes to determine the effects of the fish removals from the ecosystem. The lakes are listed in Table 33 along with notes on their presumed condition as provided by Duane Shodeen, Metro Area Fisheries Manager. Each lake was sampled four times between June 12 and August 30, 1979. Measurements made included Secchi disc transparency; temperature and dissolved oxygen (by probe); chlorophyll, algae, and total phosphorus (upper 3 meters by tube integrator); and zooplankton (vertical haul 17 cm Wisconsin net). Recording echo sounder scans were done to evaluate the size of the fish population.

CHARACTERISTICS OF THE LAKES

Because very little previous data was available for most of these lakes, a direct comparison with earlier years was not possible for most. Rather than before and after comparisons therefore, we looked for characteristics common to winter-kill lakes. Specifically, we were interested in:

1. Zooplankton populations -- do winter-kill lakes have different and/or larger herbivores?
2. Chlorophyll/TP ratios -- are they lower in lakes with larger herbivores?
3. Transparency -- is it generally greater in winter-kill lakes?
4. Algal populations -- are they different in winter-kill lakes?

Because test nettings were not done in all of the lakes, and because the echo sounder malfunctioned, we do not actually know which lakes had complete kills or which had partial or no kills. Therefore there is a danger of circularity in the interpretation. Nevertheless, it is instructive to analyze the results, which are summarized in Table 34.

Zooplankton

Of the eight lakes suspected of hard winter-kill, (Table 33) three of them, Rebecca, Hydes, and Powderhorn were dominated by *Daphnia pulex* to the virtual exclusion of all other cladocerans. Although Rebecca and Hydes did have diaptomids present, they were absent from Powderhorn Lake. O'Dowd Lake had *Daphnia pulex* on one date when it shared dominance with *Daphnia galeata mendotae*. The lake had, in addition to *Diaptomus*, *Ceriodaphnia* and *Diaphanosoma*. Thus four of the eight suspected hard-kill lakes had *D. pulex* in them, and in three of the lakes these were the dominant form. Furthermore, in these three the *Daphnia pulex* were abundant, averaging 19-33 1-1 throughout the water column. In the four remaining suspected hard-kill lakes the main species of *Daphnia* were *D. retrocurva*, *D. parvula* or *D. galeata*.

In the lakes suspected of only partial or no winter-kill the main species of *Daphnia* were *D. galeata* and *D. retrocurva*. However in four of the lakes, *D. pulex* made its appearance -- as a co-dominant in Sarah Lake and on one date in Bass Lake; and as the sole dominant in Ryan Lake, at a concentration of 2 1-1 and in Cedar Lake at a concentration of 20 1-1. Cedar Lake was listed as a control but Duane Shodeen had noted that it was low in panfish. Thus in four lakes suspected of hard winter-kill, in two lakes suspected of partial winter-kill, and in one lake known to be low in panfish *D. pulex* was the dominant or abundant. This situation presumably resulted

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*by Joseph Shapiro

Table 33: Lakes examined in winterkill study.

Lake	County	Comments
Presumed "Hard-Kill" Lakes		
Rebecca	Hennepin	infrequent kills
Snail	Ramsey	infrequent kills
O'Dowd	Scott	killed in '75 back to capacity
Thole	Scott	killed in '75 back to capacity
Eagle	Carver	frequent recent kills
Hydes	Carver	infrequent kills
Sunset	Washington	killed in '75
Powderhorn	Hennepin	
Presumed "Partial-Kill" Lakes		
Owasso	Ramsey	
Josephine	Ramsey	
Bass	Hennepin	
Ryan	Hennepin	
umed No Kill Control Lakes		
Sarah		
Turtle		
Cedar		low in panfish, aerated in '79
Fish		high in panfish
Bavaria		
Pierson		
Clear		

from the reduction of fish predation. Only in Sarah Lake was the presence of D. pulex unexpected.

The case of Eagle Lake is unique. Although it had a consistently large population of Daphnia, averaging 54 l^{-1}, the only species present were D. galeata and D. parvula. Thus the size of the population suggests that the lake did winter-kill but the species suggest otherwise. The average number of Daphnia among the other lakes, omitting those seven in which D. pulex dominated, was 9 l^{-1}.

Chlorophyll/phosphorus Ratios

Many of the data in this report suggest that Daphnia, especially D. pulex, are effective in reducing algal populations. Accordingly, it is of interest to compare chlorophyll/phosphorus ratios in the lakes containing many Daphnia with the ratios in the other lakes. Among the four lakes having D. pulex as dominant and in abundance, Rebecca, Hydes, Powderhorn, and Cedar, the mean chlorophyll/TP was .132 ± .046. If the single exceptionally high values (Table 34) for Rebecca, Hydes, and Powderhorn are omitted, the ratio is even lower, .064 ± .030. Among the remaining fifteen lakes chl/TP was .362 ± .136. Interestingly, Eagle Lake, despite its great abundance of D. galeata and D. parvula, had a high chlorophyll/TP ratio (.313). The data suggest, therefore, that the lakes with predominantly D. pulex have significantly lower algal crops than the other lakes. This shows up well when the data are plotted as chlorophyll vs. phosphorus in Figure 79. The D. pulex lakes fall on a line very different in slope from those lakes having other species of Daphnia.

Transparency

The transparency of a lake is determined only in part by the chlorophyll content of the water. Nevertheless, comparison of the four D. pulex lakes with the other fifteen lakes shows the former to have a mean Secchi disc transparency of 2.07 ± .57 m (2.30 ± .82 m if the single exceptionally low values for Rebecca and Powderhorn lakes are omitted) as compared with 1.63 ± .68 m for the non-D. pulex lakes. Again Eagle Lake, despite its large population of Daphnia has a low transparency (0.57 m).

A limited amount of earlier transparency data was available for direct comparison as six of the lakes had been involved in a Secchi disc survey within the period 1973-1978. These lakes are compared for the period July-August in Table 35. The three "control" lakes, Sarah, Turtle, and Cedar had very similar transparencies in both periods suggesting that their herbivore populations were stable. The three "partial winter-kill lakes", Owasso, Ryan, and Bass, doubled in transparency. As noted earlier, Ryan and Bass did have low concentrations of D. pulex, but Owasso had none. Data for Bass and Owasso are lacking, but observations on Ryan Lake in 1977 and 1978 showed Daphnia virtually absent from the lake.

Algae

The algal samples have not been investigated in detail, but certain observations have been made. In particular we have been interested in the occurrence of so-called "flake" blooms of Aphanizomenon and, as such flakes tend to break apart during preservation, samples of water were also filtered through glass fiber filters to be used for assessment of the abundance of the flakes. The results of these filtrations are shown in Table 36. Although

Table 34: Chemical and biological data for lakes examined in the winterkill study. *Daphnia* species are: P = pulex; R = retrocurva; G = galeata; V = parvula; u = small unknown, not pulex.

Rebecca

	6-27	7-20	8-8	8-30
T.P.	106	37	63	205
Chl.*a*	33.5	3.4	5.1	25.0
S.D.	.94	4.60	4.11	2.29
D.O.	.10	.05	.05	
Daph. sp.	RP	PG	P	P
Daph. #	38	13	11	14
Chl/TP	.32	.09	.08	.12

Snail

	6-13	7-4	7-27	8-13
T.P.	33	35	31	37
Chl.*a*	7.5	9.9	9.5	13.1
S.D.	4.05	2.29	2.16	2.29
D.O.	.10	.30	.10	
Daph. sp.	RG	RG	u	u
Daph. #	26	5	3	.3
Chl/TP	.23	.28	.31	.35

O'Dowd

	6-25	7-18	8-6	8-29
T.P.	60	62	110	147
Chl.*a*	29.9	31.1	89.0	118
S.D.	.98	1.01	.61	.61
D.O.	.50	.05	.05	
Daph. sp.	G	GP	G	u
Daph. #	28	8	6	5
Chl/TP	.49	.50	.81	.80

Thole

	6-25	7-18	8-6	8-29
T.P.	114	175	172	162
Chl.*a*	58.3	50.5	91.1	96.9
S.D.	.88	.64	.55	.43
D.O.	3.0	.10	.20	
Daph. sp.	u			
Daph. #	1	0	0	0
Chl/TP	.51	.29	.53	.60

Eagle

	6-18	7-13	8-3	8-22
T.P.	315	301	427	447
Chl.*a*	69	71	193	151
S.D.	1.07	.52	.27	.43
D.O.	.10	.10	.10	
Daph. sp.	u	VG	G	GV
Daph. #	128	39	18	29
Chl/TP	.22	.24	.45	.34

Hydes

	6-18	7-13	8-3	8-29
T.P.	450	486	522	582
Chl.*a*	1.6	104	51	22
S.D.	2.67	2.29	1.22	2.32
D.O.	5.6	.10	.05	
Daph. sp.	P	P	P	P
Daph. #	49	17	46	21
Chl/TP	.004	.21	.10	.04

T.P. = total phosphorus mg m^{-3}; Chl *a* = chlorophyll mg m^{-3}
S.D. = Secchi disc transparency, m; D.O. = dissolved oxygen mg l^{-1}
Daph. # = # of *Daphnia* per liter of the whole water column

Table 34: Continued

Sunset

	6-21	7-8	7-30	8-21
T.P.	161	78	104	82
Chl.a	23.3	20.2	61.7	38.4
S.D.	2.35	1.55	.79	1.22
D.O.	7.0	5.1	8.2	
Daph. sp.	G	G		u
Daph. #	114	6	0	.5
Chl/TP	.14	.26	.59	.47

Josephine

	6-13	7-3	7-27	8-13
T.P.	37	59	55	65
Chl.a	6.7	11.1	14.8	3.6
S.D.	2.29	1.34	.94	1.34
D.O.	.01	.10	.05	
Daph. sp.	GR	GR	RG	RG
Daph. #	14	2	2	3
Chl/TP	.18	.19	.27	.06

Powderhorn

	6-21	7-8	7-30	8-21
T.P.	283	310	376	133
Chl.a	2.9	225	8.0	4.7
S.D.	2.56	.91	1.49	1.71
[illegible]	1.1	0.4	.05	
[illegible] sp.	P	P	u	P
Daph. #	24	32	.03	32
Chl/TP	.01	.73	.02	.04

Bass

	6-27	7-20	8-8	8-30
T.P.	40	54	73	109
Chl.a	4.2	19.0	29.4	58.5
S.D.	4.51	1.83	1.28	.85
D.O.	.10	.05	.05	
Daph. sp.	G	GP	u	u
Daph. #	30	7	4	5
Chl/TP	.11	.35	.40	.54

Owasso

	6-13	7-3	7-27	8-13
T.P.	60	62	79	91
Chl.a	13.4	8.0	9.5	14.7
S.D.	2.90	3.72	2.47	1.86
D.O.	.10	.20	.05	
Daph. sp.	GR	G	GR	u
Daph. #	26	13	.6	1
Chl/TP	.22	.13	.12	.16

Ryan

	6-27	7-20	8-8	8-30
T.P.	139	104	108	122
Chl.a	13.4	19.8	38.9	39.9
S.D.	2.56	1.52	1.10	1.10
D.O.	.05	.05	.10	
Daph. sp.	P	P	u	u
Daph. #	7	1	.5	.5
Chl/TP	.10	.19	.36	.33

Table 34: Continued

Sarah

	6-27	7-20	8-8	8-30
T.P.	123	89	75	125
Chl.a	12.5	47.3	53.2	120
S.D.	3.32	.79	.82	.79
D.O.	.10	.05	.01	
Daph. sp.	PG	PG	G	PG
Daph. #	8	2	2	2
Chl/TP	.10	.53	.71	.96

Fish

	6-25	7-18	8-6	8-29
T.P.	44	35	46	47
Chl.a	22.6	16.2	27.6	27.2
S.D.	1.49	1.19	.88	1.25
D.O.	.05	.05	.05	
Daph. sp.	G	RG	R	R
Daph. #	7	1	3	17
Chl/TP	.51	.46	.60	.58

Turtle

	6-13	7-4	7-27	8-21
T.P.		24	20	23
Chl.a		5.6	4.7	9.4
S.D.		2.01	1.98	1.68
D.O.		3.9	.10	
Daph. sp.		R	R	RG
Daph. #		5	4	8
Chl/TP		.23	.24	.41

Bavaria

	6-18	7-13	8-3	8-22
T.P.		48	33	
Chl.a		27.5	3.4	
S.D.		1.07	3.47	
D.O.		.20	.05	
Daph. sp.		GR	RG	
Daph. #		2	5	
Chl/TP		.57	.10	

Cedar

	6-25	7-18	8-6	8-29
T.P.	443	562		654
Chl.a	59.2	44.1		37.9
S.D.	1.52	1.58		1.43
D.O.	7.4	2.1		
Daph. sp.	P	P		P
Daph. #	10	46		4
Chl/TP	.13	.08		.06

Pierson

	6-18	7-13	8-3	8-22
T.P.	32	28	27	25
Chl.a	13.4	6.2		7.8
S.D.	2.04	2.32	2.71	2.53
D.O.	6.7	.25		.05
Daph. sp.	G	G	RG	RG
Daph. #	5	3	6	2
Chl/TP	.42	.22		.31

Clear

	6-21	7-8	7-30	8-21
T.P.	89	62	45	60
Chl.a	28.7	12.6	10.8	30.1
S.D.	.98	1.16	1.52	1.25
D.O.	5.0	.05	.05	
Daph. sp.	G	GR	GR	RG
Daph. #	22	3	7	6
Chl/TP	.32	.20	.24	.50

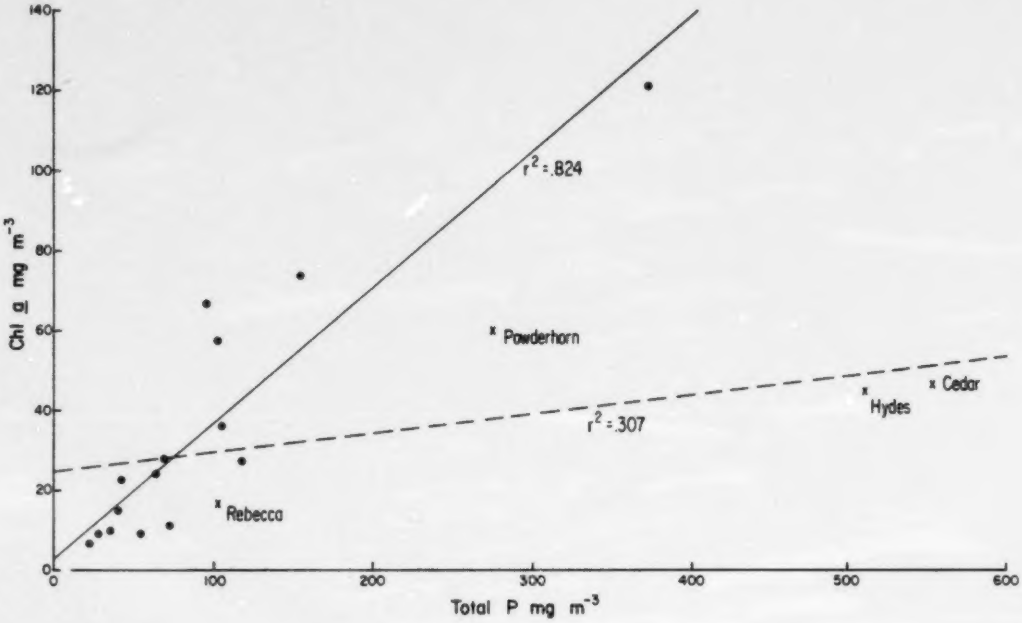

Fig. 79. Chlorophyll/TP relations in lakes with (X) and without (·) abundant *Daphnia pulex*.

Table 35: Secchi disc transparencies of partial winter-kill and control lakes for the period 1973-1978, and for 1979, following winter-kill: Values are July-August means.

	Transparency (m)	
	1973-78	1979
Control Lakes		
Sarah	0.99	0.80
Cedar	1.22	1.51
Turtle	1.83	1.90
Partial-Kill Lakes		
Ryan	0.61	1.24
Bass	0.69	1.32
Owasso	1.33	2.68

Table 36: Abundance of Aphanizomenon flakes in the lakes containing them in 1979. 0 = absent, 1 = very sparse, 2 = moderate, 3 = very abundant.

	Sampling period (see Table 2 for date)			
Lake	A	B	C	D
Pierson	0	0	0	1
Sarah	1	0	0	0
Thole	1	0	0	0
Hydes	0	2	3	2
Powderhorn	1	3	2	2
Cedar	3	2	-	1

six of the nineteen lakes had some flakes during the sampling period, only three of them had flakes in abundance. Interestingly, these three lakes, Hydes, Powderhorn, and Cedar were three of the four lakes that were dominated by abundant *Daphnia pulex* (means = 33, 22, and 20 l-l respectively). The fourth such lake, Rebecca, in which *D. pulex* averaged 19 l-l, had no flakes. However, Sarah Lake which had a few flakes early in the season also had *D. pulex*.

The lakes characterized by abundant flakes were also those with (except for Eagle Lake) the highest total phosphorus - 510, 278, and 553 ug l-l respectively. Again Rebecca Lake had a low value -- 103 ug l-l. Eagle Lake, despite its high total phosphorus and its abundance of *Daphnia* (54 l-l) had no flakes. However, the *Daphnia* were, as noted, *D. galeata* and *D. parvula*, not *D. pulex*. Thus lakes with abundant flakes appear to be characterized by abundant *D. pulex* and high total phosphorus.

Finally, as noted by Lynch (1980), dissolved oxygen may play a role in formation of flakes by *Aphanizomenon*. Of the four lakes with many *D. pulex*, all but Rebecca had significant amounts of dissolved oxygen near the bottom during the first three quarters of the sampling period -- specifically, Hydes, 1.9 mg l-l; Powderhorn, 0.52 mg l-l; Cedar, 4.8 mg l-l. Rebecca, on the other hand, had only 0.07 mg l-l.

SUMMARY

In summary, lakes which winter-kill or are otherwise low in panfish develop large populations of *D. pulex* leading to low Chl/TP ratios and high transparencies. These lakes may also have *Aphanizomenon* in the form of large flakes. The presence of the flakes may be attributed to high concentrations of TP in the water and to adequate dissolved oxygen near the sediments.

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Lynch, M. 1980. *Aphanizomenon* blooms: alternate control and cultivation by *Daphnia pulex*. *In* Evolution and Ecology of Zooplankton Communities. Special Symp. Vol. 3. ASLO. W.C. Kerfoot, (ed). Univ. Press of New England, Hanover, N.H.

IV.D. EFFECTS OF WINTERKILL IN LAKE OF THE ISLES*

This lake lies within the city limits of Minneapolis and is administered by the Minneapolis Park and Recreation Board. It is 41.3 ha in area, has a mean depth of 2.7 m, and is connected on the west to Cedar Lake and on the south to Lake Calhoun. Lake of the Isles has been described in detail by Shapiro and Pfannkuch (1973) and Shapiro (1974). In essence it is highly eutrophic, receiving a phosphorus loading of about 2 g m^{-2} yr^{-1}, virtually all from urban runoff. In 1930-31, although the lake had blue-green algae in it, they were not as abundant as at present and Ceratium formed a large proportion of the biomass. The Minnesota Department of Natural Resources records show that in July 1927, the Secchi disc transparency was about 2.5 m. In the past few years, it has been as low as 0.4 m. Daphnia pulex and Diaphanosoma were the only two cladocerans reported in 1930-31 (Klak 1933). In recent years Daphnia pulex has been scarce, and there are now 9-11 species of cladocerans present -- all, except for the few Leptodora, smaller in size than D. pulex.

The Park Board hopes to restore the lake by diversion of nutrients from it through use of first flush storm water diverters. An EPA lake restoration grant testing the idea was implemented beginning in 1979.

This section describes the effects of a winter-kill that occurred during the winter of 1976-1977.

Chlorophyll

Surface chlorophyll concentrations from 1971-1978 are shown in Figure 80. (Where they have been taken, depth integrated samples compared very closely.) In general chlorophyll is low in May and especially in June, increases throughout July to a peak in August, and begins to decline before the end of August to a lower value during September. Concentrations in 1977-78 were lower than during the preceding six years.

Algal Species

Normally blue-green algae are relatively scarce during May and June but become abundant in July and August. In fact blue-greens may become so scarce in spring and early summer as to be undetectable. In place of the blue-greens are usually found a variety of apparently ungrazable greens. In 1975 Sphaerocystis dominated beyond July 1, and in 1976 Oocystis and Sphaerocystis co-dominated with the few blue-greens. These were accompanied by numerous other greens such as Scenedesmus, Eudorina, Tetraedron, Cosmarium, Pandorina, and Chlorella. The situation was somewhat different in 1977. On May 20, although there were no blue-greens there were also no large ungrazable greens, the algal population consisting exclusively of Chlamydomonas, Schroederia, green microflagellates, cryptomonads, and Euglena. By June 15 there were a very few filaments of Anabaena flos-aquae, and Oscillatoria limnetica, but the same green algae were present. A few diatoms had appeared and Ceratium hirundinella, which had been found in moderate numbers in earlier years was present in low numbers.

Ceratium continued to increase in abundance reaching a high of 1800 ml^{-1} and making up by far the bulk of the algal biomass on August 1, 1977, coincident with the maximum abundance of blue-greens which had been increasing. Cryptomonads were more abundant in 1977, being in excess of 1500 ml^{-1} until early July. Although Aphanizomenon flos-aquae became abundant in the lake during August, it did not form so-called grass blade blooms nor had it in previous years.

On July 19, 1977, when transparency was 2.4 m, a study was made of the vertical distribution of the algae. The results for the most important

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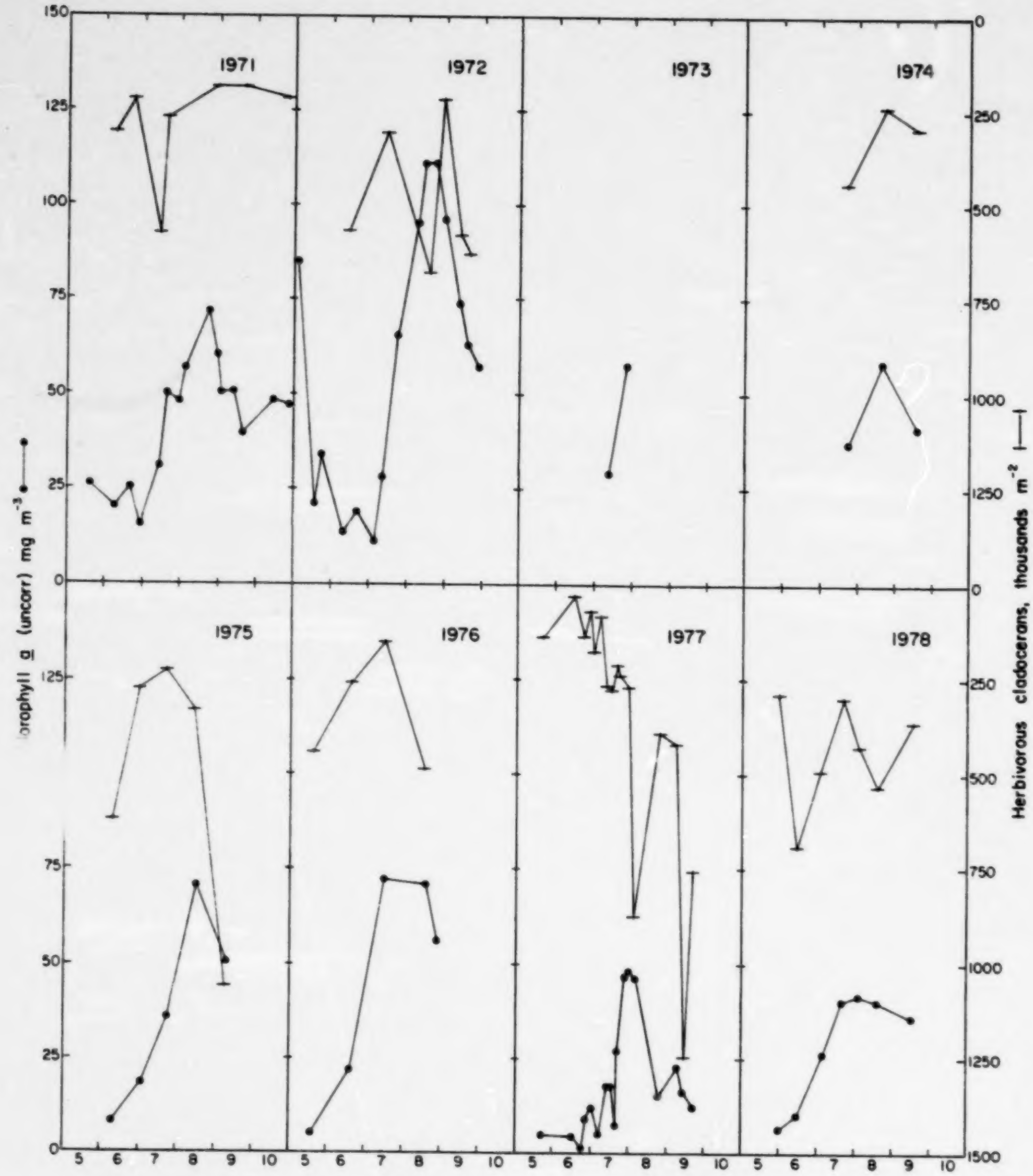

Fig. 80. Concentrations of chlorophyll (·) in surface waters and cladocerans (-) in the water column, L. of the Isles, 1971 - 1978.

species are shown in Table 37. The surface sample was lost. Note the different distributions. Both species of *Oscillatoria* were more abundant at intermediate depth, as was *Ceratium*. *Merismopedia* was virtually absent in the upper 4 meters of the water column but was very abundant at 5 meters. The green microflagellates on the other hand were found throughout the 5 m depth but were especially abundant in the upper 2 m.

Transparency

Transparencies in Lake of the Isles have ranged from as low as .40 m to as high as 4.2 m (Fig. 81). In general summer, i.e. July, August, values, are less than 1 m but seem to have increased gradually from 1971 and 1972 to 1975 when they again declined. The 1977 season is clearly different with transparencies always exceeding 1 m and in fact exceeding 2 m except for a 1-month period from late July to late August. It is also clear from a comparison of the shapes of the Figuress (80 and 81) that there is a close relationship between transparency and chlorophyll in Lake of the Isles. Regression of 1/Secchi disc against chlorophyll for all data of 1972-1978 (n = 67) yields an r2 value of 0.656. Regression of only the 1977 data yields an r2 value of 0.858. Thus the high transparencies in 1977 did result from low algal populations.

Total Phosphorus

Total phosphorus has been measured in the lake since 1971. Surface concentrations shown in Figure 82 follow a curious pattern. In 1971-72 they averaged about 90 ug 1-1 but from 1973-1975 the concentrations were lower at about 60-70 ug 1-1. In 1976 concentrations were a bit higher -- 70-80 ug 1-1. They were lower again in 1977, about 50-60 ug 1-1 and rose in 1978 to very high concentrations -- up to 210 ug 1-1. There have been no events taking place in the watershed which could explain these changes, but examination of rainfall records may shed some light. From 1970 to 1974 precipitation declined continuously from 30.5 in yr-1 to 19.1 in yr-1. As the lake depends on runoff for its phosphorus load and the average concentration of runoff to Lake of the Isles is in excess of 600 mg m-3 (Shapiro and Pfannkuch 1973) a shortage of this runoff could explain the decline in concentration. Unfortunately this relationship breaks down, as, in 1975 when rainfall reached the high level of 35.15 in yr-1, total phosphorus concentration in the lake did not change appreciably. Furthermore 1976, when concentrations rose somewhat above those in 1975, was an especially dry year, with only 16.5 inches of precipitation. Finally 1977 (34.9) and 1978 (27.5+) do not fit the relationship either. In particular the 1978 (27.5+) values lack an explanation. A complicating factor in this lake is the large carp population. These fish, which are capable of pumping phosphorus from the sediments (Lamarra 1975), were abundant in the lake in 1975 but many died off in winter 1976-77. At this time therefore the phosphorus concentrations cannot be explained satisfactorily.

Dissolved Oxygen

Anoxia in the bottom waters of Lake of the Isles has been detected as early as May 23, 1972 and generally by mid- to late-July dissolved oxygen has disappeared entirely from the hypolimnion. Some differences from year to year do occur, however, as shown in Figure 83.

Zooplankton

The zooplankton of Lake of the Isles is diverse with at least 11 cladocerans, 4 copepods and about a dozen rotifers (Table 38). In 1933 there

Table 37: Vertical distribution of the most abundant algae in Lake of the Isles July 19, 1977. (numbers of individuals or filaments)

Depth (m)	Oscillatoria Agardhii	Oscillatoria tenuis	green microflagellates	Merismopedia tenuissima	Ceratium hirundinella
0	--	--	--	--	--
1	36	72	324	0	135
2	54	171	225	0	315
3	54	414	117	9	2000
4	324	324	126	0	729
5	99	9	90	4500	315

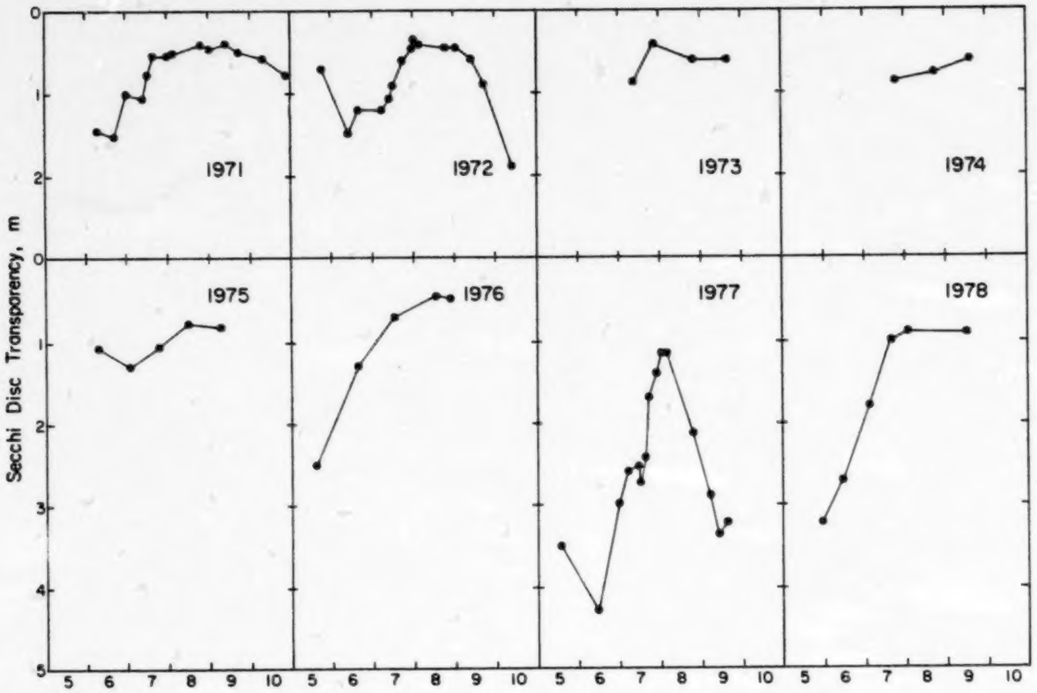

Fig. 81. Secchi disc transparencies in L. of the Isles, 1971 - 1978.

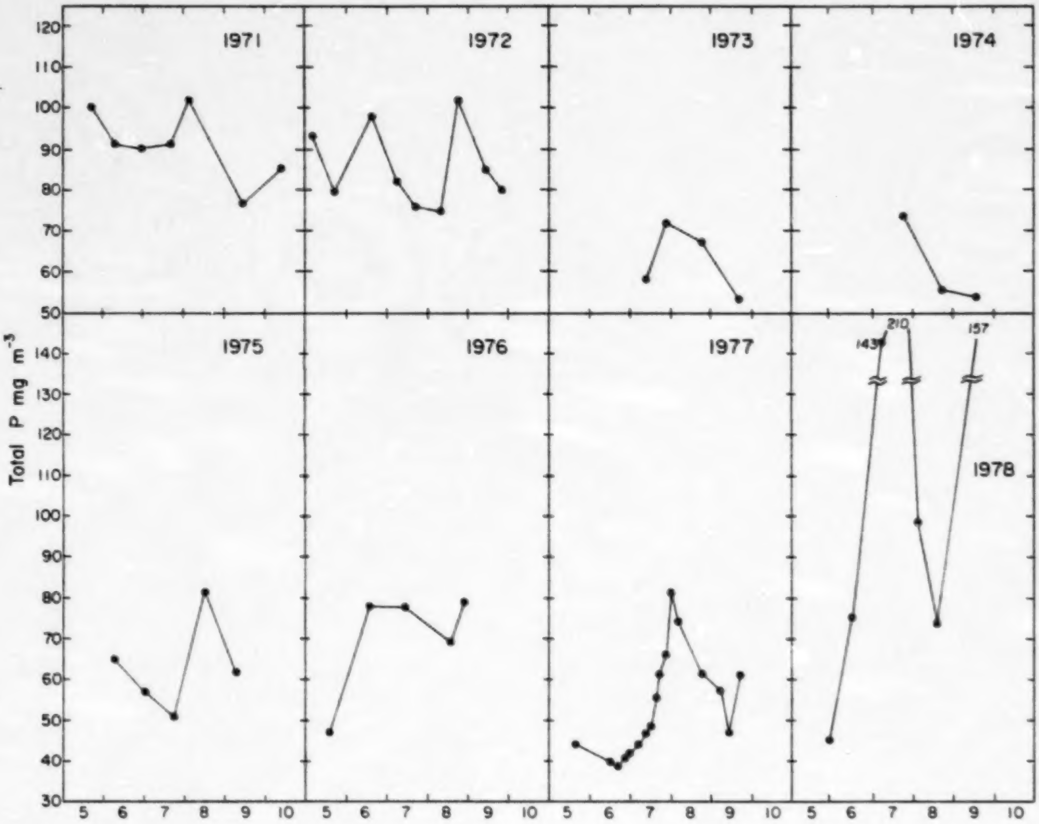

Fig. 82. Concentrations of total phosphorus in surface waters of L. of the Isles, 1971 - 1978.

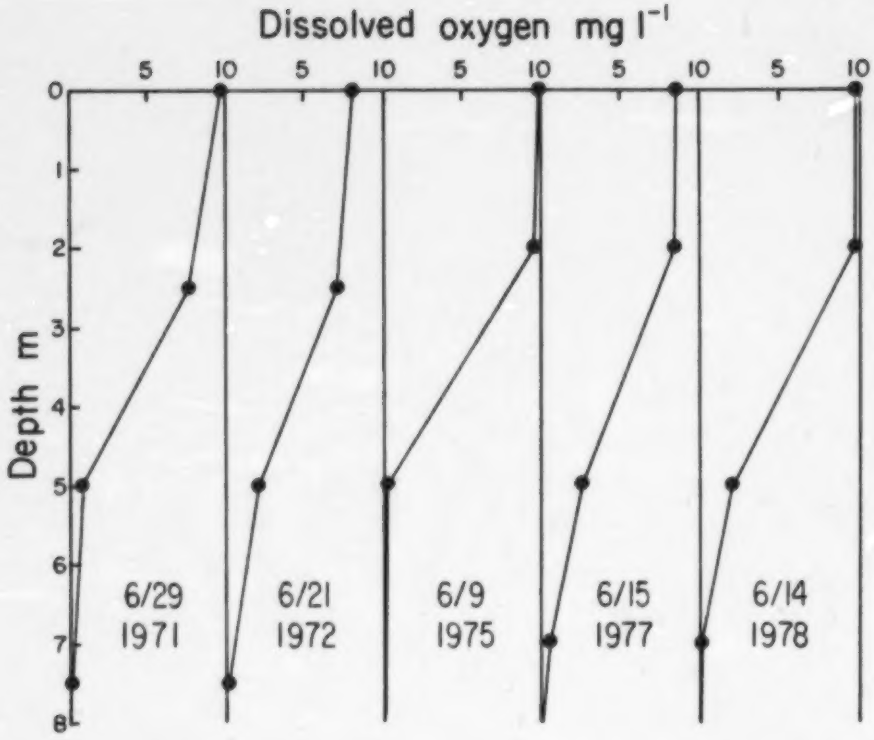

Fig. 83. Profiles of dissolved oxygen in L. of the Isles on approximately the same date in June for five years.

Table 38: Lake of the Isles Zooplankton (thousands · m-2).

	1971							1972						
	June		July		Aug	Sept	Oct	May	June	July	Aug		Sept	
	8	23	14	21	31	21	28	18	13	13	18	29	13	20
CLADOCERA:														
Daphnia pulex														
Daphnia galeata	120	83	11	15	9	7	176		148	5	22			62
Daphnia retrocurva	147	92	23	117	42	87	27		148	1	101			198
Daphnia parvula										3	7			
Daphnia ambigua	4	5							114	23				
Diaphanosoma leuctenbergianum	31	28	170	68	79	58	5		38	32	86	150	260	110
Ceriodaphnia lacustris	4	9	370	57	61	40	14		120	240	460	71	320	260
Total "grazers"	306	217	574	257	191	192	222	0	568	304	676	221	580	630
Bosmina longirostris	1905	18	306	887	108	918	1817	42	1900	140	250	610	2400	270
Chydorus sphaericus	89	28	272	279	84	69	578	21	13	59	220	120	510	730
Leptodora kindtii													16	5
Pleuroxus sp.														
COPEPODA:														
Cyclops bicuspidatus	333	9	5	169	7	174	438	--	--	--	--	--	--	--
Cyclops vernalis	9	18		15	22	58	44	--	--	--	--	--	--	--
Diaptomus siciloides	9	5	11	26		7	18	--	--	--	--	--	--	--
Nauplii	400	777	687	49	500	687	511	1500	800	930	1100	980	1700	820
Mesocyclops edax	44	262	158	77	262	163	44	--	--	--	--	--	--	--
ROTIFERA:														
Asplancha priodonta	9			19	19	11	5	--	--	--	--	--	--	--
Conochiloides sp.	4			15	3541	36	23	--	--	--	--	--	--	--
Keratella cochlearis	382	64	87	8	206	195	1288	--	--	--	--	--	--	--
Keratella quadrata	626	32					50	--	--	--	--	--	--	--
Kellicottia longispina	4			8	14	29	285	--	--	--	--	--	--	--
Polyarthra vulgaris	297	184	283		136	69	330	--	--	--	--	--	--	--
Trichocerca multicrinis					93			--	--	--	--	--	--	--
T. similis								--	--	--	--	--	--	--
Lepadella sp.								--	--	--	--	--	--	--
Mytilinia sp.								--	--	--	--	--	--	--
Filinia sp.					196	4		--	--	--	--	--	--	--
Monostyla sp.							14	--	--	--	--	--	--	--
Platyias patulus								--	--	--	--	--	--	--
Testudinella patina								--	--	--	--	--	--	--
Euchlanis dilata								--	--	--	--	--	--	--

Table 38 Continued

	1974			1975					1976				1977				
	July	Aug	Sept	June	July		Aug	Sept	May	June	July	Aug	May	June			
	22	20	16	9	1	23	15	9	18	18	15	17	20	15	23	28	30
CLADOCERA:																	
D. pulex														1	31	19	39
D. galeata	82	28	156	331	68	22	25	65	68	155	28	14	120	27	107	45	128
D. retrocurva	17	52	112	214	25	19	50	827	272	54	19	77	2			4	4
D. parvula	8		18	34	3	6		3	8				13				
D. ambigua									94								
D. leuctenbergianum	297	156	4	5	167	174	233	124		44	107	395					5
C. lacustris	42		8	37	18	6	25	41				7					
Total "grazers"	446	236	298	621	281	227	333	1060	442	253	154	493	135	28	138	68	176
B. longirostris		48	189	380			6	140	700	74		19					
C. sphaericus	328	256	55		3	407	308	385		208	512	72	2		6	2	1
L. kindtii	3													1		1	
Pleuroxus sp.																	
COPEPODA:																	
C. bicuspidatus	8	14	33	825		11	9	29	1046			51	420			2	
C. vernalis	149	369	166	69	51	41	68	113			25	47			11	2	
D. siciloides	173	73	17	190	170	112	133	51	205	94	38	68	89	98	275	135	201
Nauplii	133	86	181	5	60	62	83	255	1233	407	667	1363	810	128	554	568	779
M. edax	66	88	20	25		104	25	49	20	27	66	166	79	70	108	65	165
ROTIFERA																	
A. priodonta	5		17	9		3	6	3									
Conochiloides sp.	8					3	2			3							
K. cochlearis	3	14	25	14	3	9		3	2337	91	244	414	58	58	332	526	1192
K. quadrata				51					38	13				2			
K. longispina	8	10				6		6	38		231	98	2				
P. vulgaris	8					3				10	47	21					
T. multicrinis							2			13	47	12	2				
T. similis																	
Lepadella sp.									8								
Mytilinia sp.											3						
Filinia sp.											117	5					
Monostyla sp.																	
P. patulus																	
T. patina																	
E. dilata																	

Table 38 Continued

	1977												1978			
	July					Aug			Sept			Nov	May	June	July	
	6	13	15	19	21	1	5	24	7	13	21	8	29	14	4	20
CLADOCERA:																
D. pulex	15	3	3		6											
D. galeata	31	6	3			6	6	52	25	33	86	195	6		3	
D. retrocurva						6	3	42	12	19	56		73	323	105	28
D. parvula									3						9	
D. ambigua											3		106	17	6	
D. leuctenbergianum	31	257	269	207	230	255	863	98	43	81	25			3	17	48
C. lacustris			3			3	3	199	333	1112	589	3	3	252	256	122
Total "grazers"	77	266	278	207	236	270	875	391	416	1245	759	198	188	595	396	198
B. longirostris						3	16	3777	133	780	336	184	54	95	26	17
C. sphaericus				3	3	10	29	52	37	24	34	2	3	24	40	187
L. kindtii																
Pleuroxus sp.																3
COPEPODA:																
C. bicuspidatus	12		3	14		3		21	65	30	15	132	221	34		
C. vernalis					3		19		31	42	22				6	
D. siciloides	225	215	314	238	130	158	159	70	97	143	142	99	209	235	145	57
Nauplii	994	788	744	521	796	661	863	899	700	651	285	5	926	704	1040	215
M. edax	108	152	191	110	97	87	115	7	43	570	268	2	36	71	71	6
ROTIFERA:																
A. priodonta				9	3	100	10	59	56	276	46	11			54	6
Conochiloides sp.															66	351
K. cochlearis	3014	1580	1219	445	899	606	745	269	136	304	290		2905	1115	165	710
K. quadrata											3		70	14	9	
K. longispina		15	24	9	109	364	500	38	6		3		85	17	17	45
P. vulgaris			3		57	77	131	1241	1292	1079	241		24	126	111	226
T. multicrinis					12	16	38	7	3	14			3			
T. similis						3										
Lepadella sp.						3							6	7	3	3
Mytilinia sp.					15	3										
Filinia sp.								17	3							
Monostyla sp.									6							
P. patulus							3									8
T. patina																31
E. dilata																

Table 38 Continued

	1978 Aug 2	 Aug 18	 Sept 15
CLADOCERA:			
D. pulex			
D. galeata	59	137	111
D. retrocurva	23		45
D. parvula	5		3
D. ambigua			
D. leuctenbergianum	147	185	50
C. lacustris	98	107	47
Total "grazers"	332	429	256
B. longirostris	33	81	26
C. sphaericus	347	465	92
L. kindtii			
Pleuroxus sp.	3		
COPEPODA:			
C. bicuspidatus			10
C. vernalis	21	55	26
D. siciloides	72	83	67
Nauplii	412	713	612
M. edax	67	44	29
ROTIFERA:			
A. priodonta			2
Conochiloides sp.	824	26	36
K. cochlearis	363	486	50
K. quadrata	3		2
K. longispina	3		2
P. vulgaris	185	63	14
T. multicrinis	5	9	
T. similis			
Lepadella sp.		2	
Mytilinia sp.			
Filinia sp.	8		5
Monostyla sp.			
P. patulus		2	
T. patina		2	
E. dilata		2	

were fewer cladocerans, with only two species, *Daphnia pulex* and *Diaphanosoma brachyuium*, reported by Klak (1933). In 1977 *Daphnia magna* was found on several occasions although it had not been found in other years. *Daphnia pulex*, although being reported in 1933, was absent in 1971-1976, but appeared in moderate concentrations (up to 39,000 m-2) in June 15-July 21 of 1977 and reappeared on two occasions in 1978. *Daphnia retrocurva* showed an inverse relationship to *D. pulex*, but *D. galeata*, generally the most abundant *Daphnia*, co-existed successfully with *Daphnia pulex* in contrast to Wirth Lake where *D. pulex* was much more abundant (IV B 2). The abundance of *D. galeata* in 1977 was no different from previous years. Certainly, however, *D. galeata* is least abundant in July and August and most abundant in June.

In addition to *D. retrocurva* being least abundant when *D. pulex* was present, *Diaphanosoma*, *Bosmina*, and *Ceriodaphnia* also showed this relationship. However, *Ceriodaphnia* was also in low abundance in 1976 so the relationship is not perfect. *Bosmina* can co-exist with *D. galeata*.

The distribution of *Chydorus sphaericus* is interesting in that it is abundant only when filamentous blue-greens abound. This is in agreement with many similar distributions in the literature and probably occurs because *C. sphaericus* moves into open water by using filaments of blue-greens as rafts. A correlation between the numbers of filaments of *Aphanizomenon flos-aquae* and the numbers of *Chydorus* yields an r value of 0.38. However it may be more significant that out of 19 pairs, in 7 where *Aphanizomenon* was rarer than 1300 filaments per milliliter, *Chydorus* was rarer than 3 m-2. Thus there may be a threshold concentration of filamentous blue-greens above which the abundance of *Chydorus* in the plankton is a function of its abundance in the littoral areas but below which it either does not become transported to the limnetic regions or only does so in small numbers.

The body sizes of the daphnids are of interest. Figure 84 shows that in May and June 1977 *D. galeata* tended to be larger than in 1976.

In addition to changes in cladocerans, *Diaptomus siciloides* was unusually abundant in 1977.

Fish

A study by the Minnesota Department of Natural Resources in 1975 showed that, whereas the bullhead, northern pike, and yellow perch populations were near the state means, the carp population was high -- above the state-wide median -- and the crappie and bluegill populations were high. During the winter of 1976-77, dissolved oxygen under the ice declined to very low levels and an estimated 95% of the fish died, including many of the carp (MDNR records). In April 1977 although there was no flow into the lake from Cedar Lake because of its low level and because the channel from Lake Calhoun was dammed off, another survey showed adult suckers, bullheads, northern pike, crappie and golden shiner but no carp. Apparently some fish had survived the winter kill, although they were few. In August 2, 1977, adult and young of the year crappie were detected along with bluegills and adult carp. The latter may have entered from Lake Calhoun when the barricade was removed, but the crappies probably resulted from spawning in the lake. There also was evidence of spawning by northern pike. Restocking of the lake took place during May 1977 when 20,000 largemouth bass fry and 45,000 walleye fry were placed in the lake; and in August 1977 when 5500 crappies , 4200 sunfish, and 14 adult northern pike removed from Wirth Lake were put into Lake of the Isles. Finally, 4300 northern pike fingerlings were added in May 1978. There was no winter-kill in the winter of 1977-78.

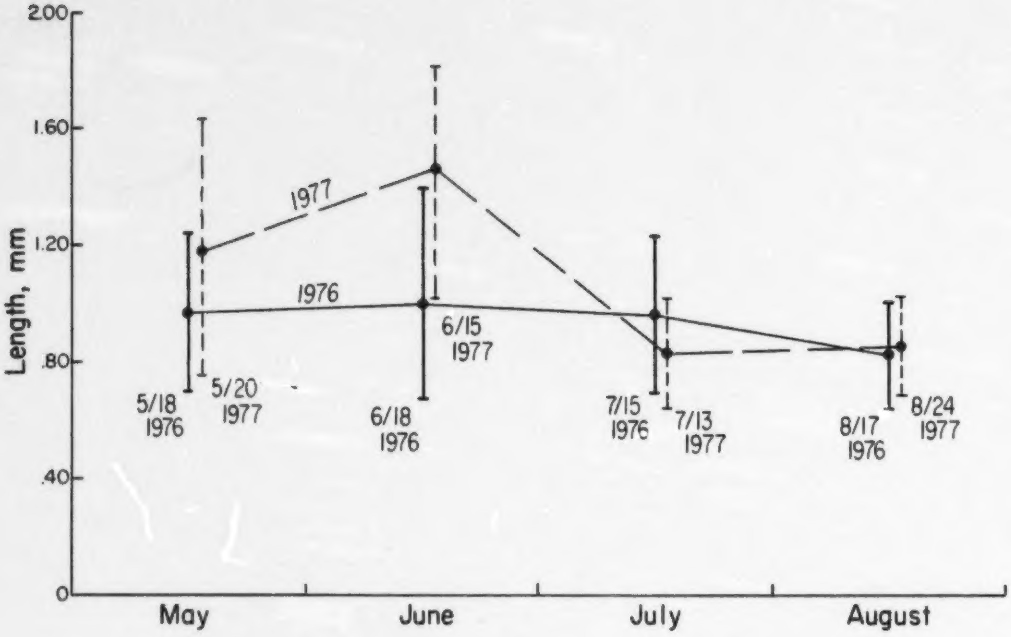

Fig. 84. Comparison of mean body lengths of Daphnia galeata in 1976 and 1977. Vertical bars are ± 1 standard deviation.

DISCUSSION

The most obvious change in Lake of the Isles during the 8-year period has been the high transparencies of 1977. So high were they that macrophyte problems -- partially caused from various *Potamogetons* -- were experienced and mechanical harvesting was done. The significant relationship in this lake between transparency and chlorophyll leaves no doubt that the cause of the high transparency was the low chlorophyll concentrations. The question is, why were the chlorophyll concentrations so low in 1977? To some extent the low algal populations probably relate to the low phosphorus concentrations i.e. while it is difficult to compare the data from year-to-year because of its nature, if the highest summer chlorophyll concentrations are compared with a visual average for total P, the correlation is good, except for 1978 when exceptionally high total phosphorus prevailed (Table 39).

That is, in 1977, total phosphorus was lowest and overall algal abundance was lowest. However it is likely that this is only part of the reason and that grazing by herbivores was also important in controlling the algal population. Thus if one compares, for each year from 1971-1976, the shape of the chlorophyll curve with the shape of the curve representing abundance of *Daphnia* plus *Diaphanosoma* plus *Ceriodaphnia*, as in Figure 80, there is a close resemblance, particularly for 1974-1975, and 1976. At the same time the 1977 data show a rather good *positive* relationship between chlorophyll and zooplankton abundance. That is, on the one hand there is evidence that low chlorophyll is caused by grazing while on the other hand the high chlorophylls of 1977 occurred with high zooplankton. This paradox may result from the fact that *Ceratium* made up 72-90% of the algal biomass in Lake of the Isles between June 15 and August 24, 1977 whereas in earlier years its contribution was much less -- averaging 27% in 1976, 8% in 1975, and 2% in 1974. Because *Ceratium* is too large to be eaten by most herbivores, its abundance probably increases when nutrients are released as other algae are consumed. Thus the positive zooplankton/chlorophyll relationship.

This situation is analogous to that in Wirth Lake where algal abundance was low in the presence of the large population of *Daphnia pulex* until the grass blade blooms of *Aphanizomenon flos-aquae* developed. It is analogous too in that the "facilitated" algae -- in Lake of the Isles, *Ceratium*, and in Wirth Lake, *Aphanizomenon flos-aquae* -- both permit greater transparency than would be expected from consideration of chlorophyll alone. This is especially true for *Ceratium* which reached its maximum abundance not at the surface but at some distance beneath it. Thus the zooplankton, both by reducing algal abundance and by fostering "large" algae, increase transparency.

Of the reason for the presence of the large grazers there can be little doubt. The fish-kill in 1976-77 was severe enough to eliminate even carp. This may also explain why total phosphorus was so low during 1977. More importantly the elimination of the planktivores removed the predation pressure from the zooplankton allowing *D. pulex*, which had not been recorded from 1971 onwards, to appear, and even allowing *D. magna*, never recorded in the lake before, to be present. Even the *D. galeata* were larger in early 1977 than at any time in 1976. Interestingly, here and in Wirth Lake, *D. galeata* was present through all of the years studied even though predation pressure was high enough to eliminate *D. pulex*

One additional feature that may have helped *Daphnia pulex* to be present in 1977, and to the extent it did in 1978, is the distribution of dissolved

Table 39: "Visual averages" of total phosphorus concentrations, and maximum chlorophyll a concentrations in L. of the Isles. 1971-1978 (mg m - 3).

Year	"Average" Total P	Maximum Chlorophyll a
1971	90	72
1972	90	111
1973	60-70	58
1974	60-70	58
1975	60-70	70
1976	70-80	72
1977	50-60	48
1978	>90	41

oxygen. As shown in Figure 83 during both of these years dissolved oxygen existed well below the thermocline of 3-5 m early in the year providing a refuge for the *Daphnia* from the few remaining warmwater fish. This situation existed in 1972 also but presumably *D. pulex* did not appear because predator pressure was still too high.

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V. THE ROLE OF PHYSICAL-CHEMICAL CONDITIONS IN AFFECTING ALGAL ABUNDANCE --LAKE HARRIET*

Lake Harriet (area 143 ha, mean depth 8.8 m) is administered by the Minneapolis Park and Recreation Board. The sole sources of water to the lake are precipitation, surface runoff, and groundwater which is known from previous studies to influence the lake (Shapiro and Pfannkuch 1973). The lake has no functional outlet. Interest attaches to this lake because of all the Minneapolis lakes it is normally the clearest and apparently the least eutrophic. Yet the phosphorus concentrations in the lake are high enough to support much larger algal populations than normally exist. The following discussion describes the probable reasons for this condition of the lake and the event that occurred in 1974, when the algal population rose to very high levels. Lake Harriet is the subject of an experiment beginning Spring 1979, in which, under an EPA lake restoration grant, the drainage area around the lake is being swept periodically to prevent nutrients from reaching the lake.

Chlorophyll

Surface chlorophyll concentrations from 1971 to 1978 are shown in Figure 85. Generally, although concentrations may be much higher in spring, they have always been below 10 mg m-3 and usually below 5 mg m-3 in summer. This pattern changed in 1974 when concentrations were always above 10 and as high as 47 mg m-3 during the summer. Concentrations in 1975 were much lower than in 1974 but still higher than usual. The data on algal biomass in Table 40 support the chlorophyll results in that 1974 had a very high algal crop and that in 1975 was still unusually high.

Phaeophytin

In 1977 phaeophytin measurements were made. The relationship between chlorophyll and phaeophytin is shown in Figure 86. High chlorophyll values are associated with high chlorophyll/phaeophytin ratios and low chlorophyll is associated with a higher proportion of phaeophytin. The r2 value is 0.63.

Algal Species

In 1971 green algae predominated through July, but from mid July the dominants were the filamentous blue greens -- Aphanizomenon and Anabaena. In 1972 the same situation occurred, but the greens persisted a few weeks longer.

In 1974 the most abundant algae were the blue greens Anabaena, Aphanizomenon, and Gomphosphaeria. In 1975 Anabaena, Aphanizomenon, and Oscillatoria abounded, in 1976 Aphanizomenon and Anabaena and in 1977 Aphanizomenon. Although Aphanizomenon flos-aquae was identified, it was never found to form "grass blade" or "flake" blooms.

Transparency

Secchi disc transparencies in Lake Harriet (Fig. 87) have ranged from a high of 5.4 m in 1972 to a low of 1.06 m in 1974. In general summer values are least with the very high transparencies occurring only in spring. The lowest July-August transparencies coincided with the high algal populations of 1974 and 1975.

Total Phosphorus

Surface concentrations of total phosphorus are shown in Figure 88. July-August values appear to have dropped from about 42 mg m-3 in 1971 to a stable level of 34-37 mg m-3 in subsequent years, except for 1974 and 1976.

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*by Joseph Shapiro

Table 40: Algal biomass in surface waters of L. Harriet, July-September, 1971-1977.

Year	Date	$mg \cdot l^{-1}$	mean
1971	7-19	4.40	
	8- 2	--	
	8-24	1.10	
	9-13	0.61	2.04
1972	7- 6	0.61	
	7-24	0.33	
	8- 9	1.10	
	8-22	1.50	
	9-13	0.70	0.85
1974	7-22	10.16	
	8-20	11.96	
	9-16	10.23	10.78
1975	7- 1	1.80	
	7-23	1.51	
	8-15	8.17	
	9- 9	2.97	3.61
1976	7-14	0.17	
	8-17	0.83	
	8-26	1.30	0.77
1977	7- 7	0.50	
	7-13	0.65	
	8- 1	2.18	
	8-24	1.75	1.27

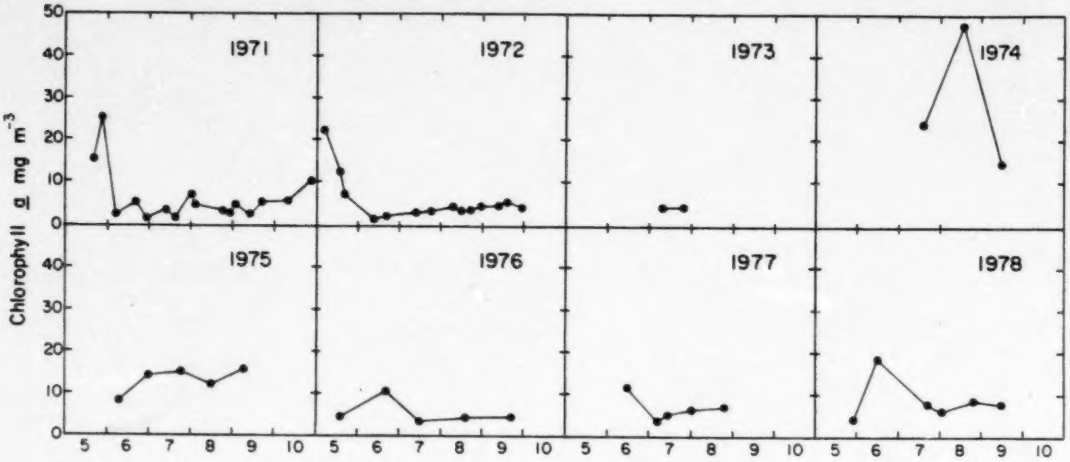

Fig. 85. Concentrations of chlorophyll in surface waters of L. Harriet, 1971 - 1978.

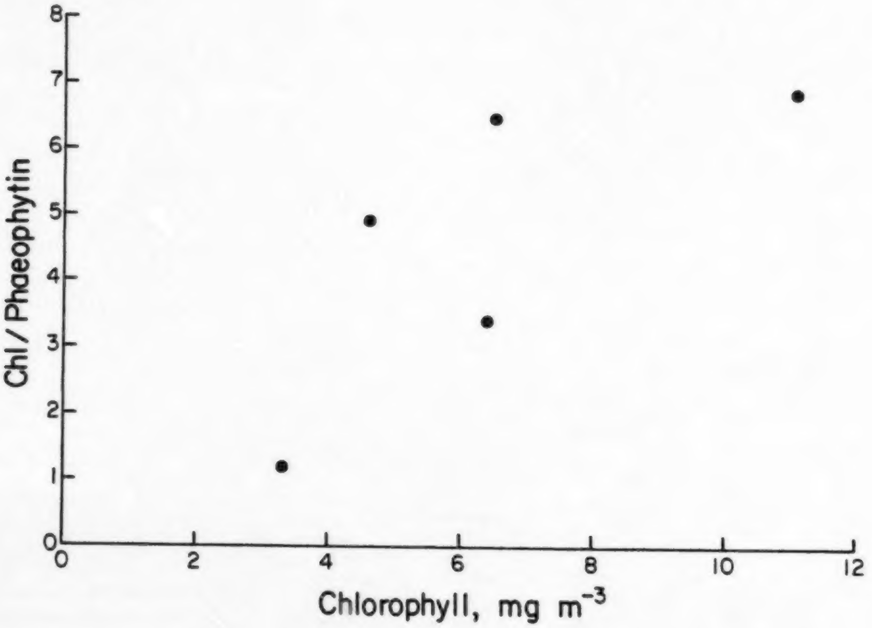

Fig. 86. Chlorophyll/phaeophytin relations in L. Harriet, 1977.

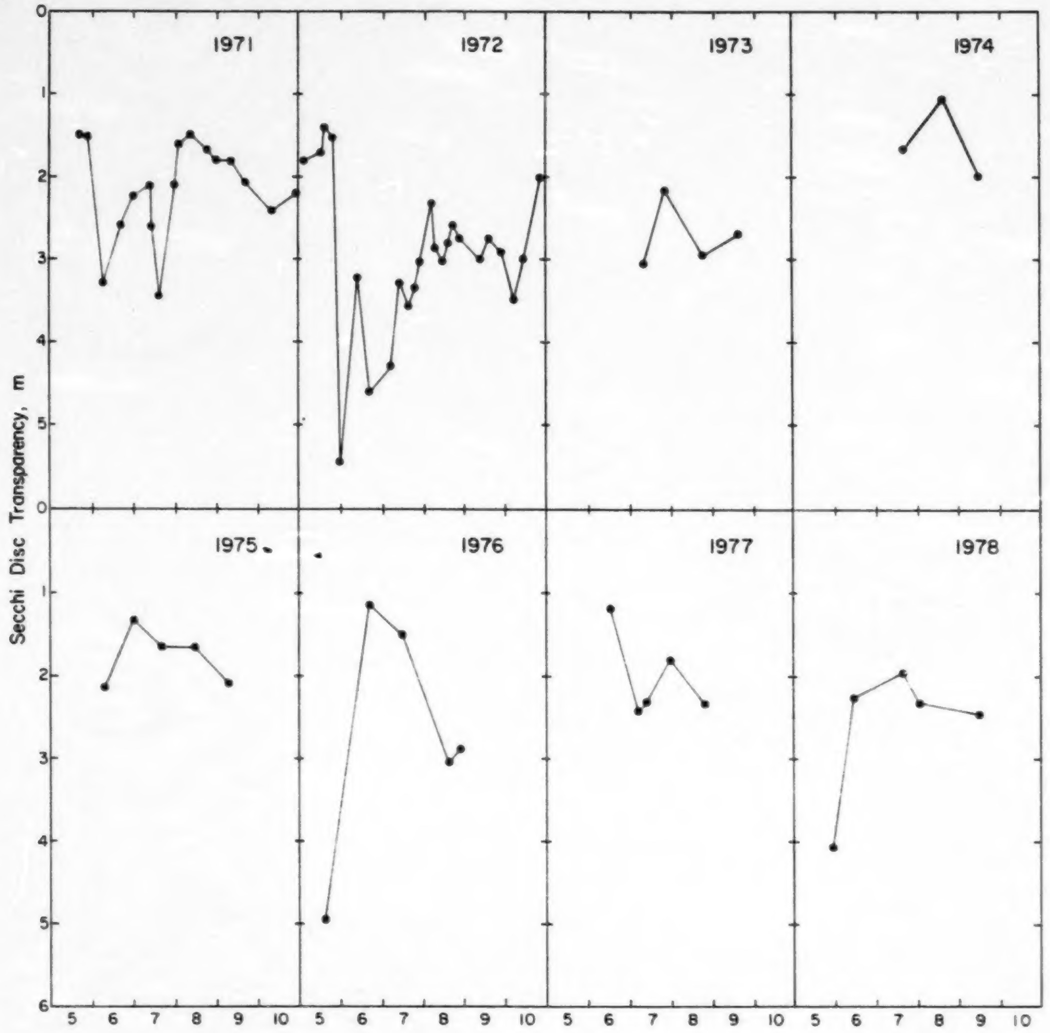

Fig. 87. Secchi disc transparencies in L. Harriet, 1971 - 1978.

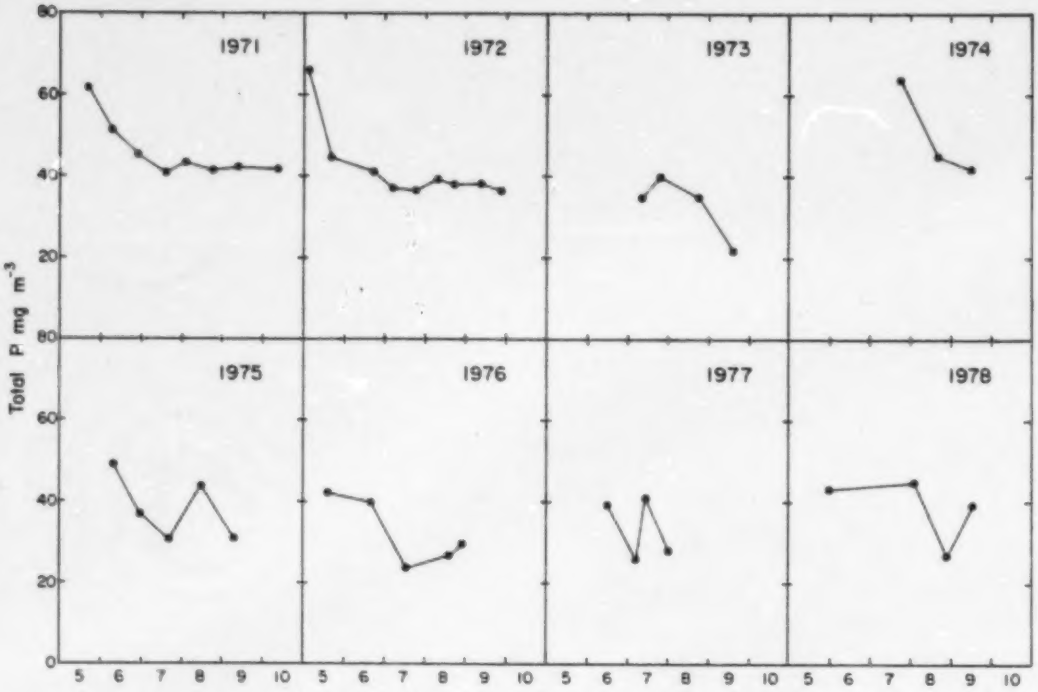

Fig. 88. Concentrations of total phosphorus in surface waters of L. Harriet, 1971 - 1978.

In 1976 values were very low probably because of the extremely low rainfall. However, in 1974 the highest July-August values occurred in spite of a very low rainfall. Thus it is unlikely that rainfall is the only determinant of total phosphorus from year to year. No explanation can be found for the high 1974 concentration.

Dissolved Oxygen

The hypolimnion of Lake Harriet becomes anoxic with the production of H2S, and the rate of depletion of oxygen does parallel the algal abundance. For example, in the period July 19-24 (Fig. 89) dissolved oxygen concentrations at 6 m were highest in 1972, intermediate in 1971 and lowest in 1974, 1975, and 1978. These correspond to 1974-75-78 having the highest chlorophyll concentrations, 1971 being next and 1972 being least. For the period August 15-26 (Fig. 89), the correlation is even better. The rank orders are:

Estimated dissolved oxygen at 6 m	Summer chlorophyll concentrations
highest 1972	lowest 1972
1971	1971
1978	1978
1975	1975
lowest 1974	highest 1974

In any event, 1974 appears to have had the least concentration of oxygen at 6 m, i.e. the rate of depletion during 1974 seems to have been the greatest, corresponding with the greatest algal abundance and the highest concentration of total phosphorus.

Zooplankton

Lake Harriet has a varied zooplankton assemblage of about 9 cladocerans, 6 copepods and 9 rotifers, as listed in Table 41. Daphnia pulex, which is of such interest in Wirth Lake and Lake of the Isles was not present in Lake Harriet in 1930-1931 (Klak 1933) and played little if any role from 1971-1978. The ones found in 1977 were immature and may not have been D. pulex. D parvula was also not abundant, but D. retrocurva was present and abundant most of the time. D. galeata, largest except for D. pulex was present and abundant, except that in 1974 it was sparse and in part of 1978 it was absent (Table 41). D. retrocurva was also sparse in 1974 but in 1978 it was perhaps more abundant than usual in the absence of D. galeata. Except for a brief appearance in 1974 and 1975, Ceriodaphnia was absent in contrast to its abundance in Wirth Lake and Lake of the Isles. Also, Bosmina, abundant in 1971 and 1972, almost disappeared thereafter. Several rotifers normally abundant were sparse during 1974 and 1975. Thus 1974 and 1975, and particularly the former, seem to have been an unusual year for the zooplankton community in Lake Harriet.

Determinations of body length of D. galeata show no significant differences in size from year to year.

Fish

A Department of Natural Resources survey on May 21-23, 1958, showed that the northern pike population was low (0.8 per net lift) and the perch

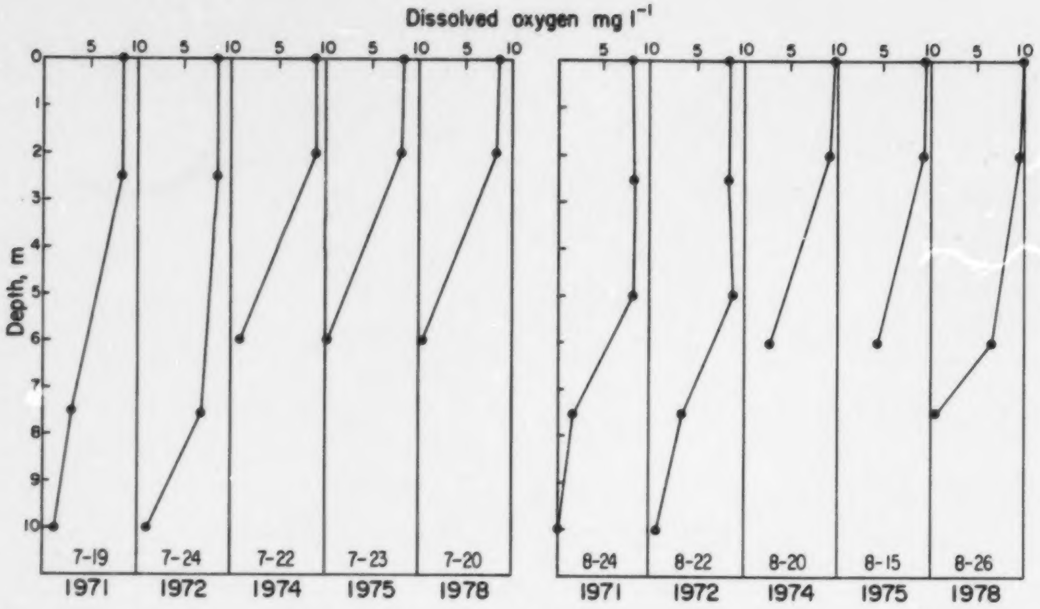

Fig. 89. Profiles of dissolved oxygen in the upper portion of L. Harriet during the month of July, 1971 - 1978, and during the month of August, 1971 - 1978.

Table 41: Zooplankton (thousands m^{-2}) in L. Harriet, 1971-1978.

	1971							1972				
	June		July		Aug.		Sept	May	June	July	Aug	
	4	23	14	30	29	31	21	19	13	13	9	18
CLADOCERA:												
Daphnia galeata mendotae	84	173	66	90	45	36	35		124	267	240	114
Daphnia retrocurva	9	87	4	56	36	40	31		23	108	94	52
Daphnia parvula	13		0	3	0	0	8		3	14	26	14
Daphnia pulex												
Total Daphnia	106	260	170	149	81	76	74	0	150	379	360	180
Ceriodaphnia lacustris												
Diaphanosoma leuctenbergianum	4	12	366	137	81	59			25	230	77	170
Bosmina longirostris	2972	215			116	20	35	33	3900	250	190	140
Chydorus sphaericus		3	4	9	163	367	31	65		28	38	170
Leptodora kindtii		3					P		P		P	
COPEPODA:												
Cyclops bicuspidatus thomasi	3551	1016	364	209	27	78	212	1300	2800	170	350	102
Cyclops vernalis						17						19
Mesocyclops edax	71	18	6	14		43						12
Diaptomus siciloides	63	77	153	189	163	142	16	65	670	390	375	290
Diaptomus oregonensis	254	51	51								125	
Diaptomus clavipes												
Nauplii	702	206	15	6	18	350	381	2300	2300	420	920	860
ROTIFERA:												
Asplanchna priodonta												
Conochilus sp.			913	1317	380	155	8	7			54	333
Kellicottia longispina	58	143	4	56		83	48	88	248	9	68	
Keratella cochlearis	409	842		6		211	90	835	2017	1099	1192	547
Keratella quadrata	960	367	12			7		180	552	114	58	30
Filinia longiseta						7	24					4
Polyarthra vulgaris	382	69				277	149	3399	4011	884	3978	935
Trichocera multicrinis		3				46	8				54	170
T. similis						30	12					

Table 41 Continued

	1972			1974			1975					1976	
	Aug	Sept		July	Aug	Sept	June	July		Aug	Sept	May	June
	29	13	20	22	20	16	9	1	23	15	9	18	22
CLADOCERA:													
D. galeata		30		32		51	31	193	4	3	0	699	119
D. retrocurva	?	0	?	5		6	66	238	63	82	16	7	
D. parvula						2	4					3	
D. pulex													
Total Daphnia	110	30	70	37	0	59	101	431	67	85	16	709	119
C. lacustris						2		13					
D. leuctenbergianum	58	85	62	340	173	259		17	63	487	5		2
B. longirostris	180							35					
C. sphaericus	280	92	110	13	37	253	4	26	192	55	17		
L. kindtii													2
COPEPODA:													
C. bicuspidatus	34	--	131			55	1006	201	21	4	21	3053	75
C. vernalis	30	--	49	33	78	366	12			4	61		
M. edax		--		6	86	183	104	81	21	15	21	10	5
D. siciloides	92	--	39	189	33	254	528	488	154	115	79	155	79
D. oregonensis	138	--	84										
D. clavipes		--	13										
Nauplii	490	--	390	18	139	369	73	144	17	19	57	978	246
ROTIFERA:													
A. priodonta		--					4	13					
Conochilus sp.		--		315	34	220		171	50	27	80		
K. longispina	48	--	18			7	4					111	38
K. cochlearis	404	--	333			4	46			1	1	410	88
K. quadrata	17	--	3				39	17				269	157
F. longiseta		--											3
P. vulgaris	547	--	348		24	4				1		7	8
T. multicrinis										1			3
T. similis													

Table 41 Continued

	1976		1977					1978					
	July	Aug	June	July		Aug		May	June	July	Aug		Sept
	15	17	15	7	13	1	24	29	14	20	2	26	15
CLADOCERA:													
D. galeata	0	43	356	75	52	26		1014	491				
D. retrocurva	112	153	9	57	26	90	38	17	52	57	162	109	40
D. parvula													
D. pulex			11?					3					
Total Daphnia	112	196	376	132	78	116	38	1034	543	57	162	109	40
C. lacustris													
D. leuctenbergianum	92	129	6	93	131	107	42		4	24	74	104	11
B. longirostris								3					
C. sphaericus	18	134	11	10		86	85	9	4	35	105	70	77
L. kindtii													
COPEPODA:													
C. bicuspidatus	9	70	413	26		13	34	416	550	3			14
C. vernalis	9	12		10			13			3	9	3	14
M. edax	53	34	40	60	22	9	4		11	84	54	18	11
D. siciloides	282	161	198	122	179	43	106	359	188	127	103	75	69
D. oregonensis													
D. clavipes													
Nauplii	561	292	531	353	467	261	344	932	639	808	692	226	204
ROTIFERA:													
A. priodonta								23	4				
Conochilus sp.	172	1177	152	6274	1509	612	102			2129	587	44	66
K. longispina	24	460	23	18	22	47	641	88	63	5	9	29	106
K. cochlearis	123	65	1211	244	205	206	518	2213	2637	480	450	174	419
K. quadrata	2		129	3				926	41	8	3		
F. longiseta	15		132						4	5	6	21	49
P. vulgaris	31	137	353	41	22	154	310	877	920	198	111	75	63
T. multicrinis		62	55	8	22	43	13				3	41	57
T. similis	2		6			4			4			10	17

population was high (168 per net lift. By 1964, after stocking with pike, pike were more abundant, nearly 3.3 per lift, while perch had declined to 28 per lift. On July 1, 1974 northern pike were at 9.3 per lift and perch had declined further to 7.3 per lift. From 1975 to 1978 a total of 1363 adult northern pike were removed as part of a program to replace them with muskellunge. Stocking of the muskellunge began in September 1974 when 295 were put into the lake and continued along with walleye stocking as follows:

Year	Muskellunge	Walleye
1974	295 adult	1300 fingerlings
1975	200 fingerlings	1000 fingerlings
1976	100 fry	1100 fingerlings
1977	102 fingerlings	1500 fingerlings
1978	100 yearlings	1100 fingerlings

The 1974 stocking with muskellunge was not successful and in 1978 observations by the DNR indicated a large perch population in the lake.

DISCUSSION

The main question to be answered is what happened in 1974? Why did the algal population increase so dramatically, as evidenced by measurements of chlorophyll, biomass and transparency. Some of the increase may have come about because, as seen in Figure 88, phosphorus was higher during 1974 than any other year from 1971 to 1978. The correlation coefficient between average surface total phosphorus values during July through September and the corresponding chlorophyll values is 0.69 (Table 42). However, if 1974 and 1975 are omitted, the r value drops to -.06. That is, the chlorophyll does not appear to be controlled ordinarily by concentrations of phosphorus. Indeed, if chlorophyll were controlled directly by phosphorus i.e. if P were the limiting factor for the algae, then the chlorophyll increase during 1974 should have been much lower. The real cause for the increase in chlorophyll seems to be that the chlorophyll/P ratio increased. This can be seen in Table 43 and Figure 90 where the chlorophyll/P ratios are shown for each year from 1971 through 1978. Only two things can account for such an increase in the ratio -- an increased supply of limiting nutrients other than P, or a decrease in the rate of grazing by the zooplankton.

The first is unlikely as no known changes occurred in the drainage area and it is difficult to understand how something such as nitrogen could increase 5 to 10-fold without an obvious change. The second explanation is very likely as the number of *Daphnia* did decrease dramatically in 1974, as seen in Table 41. Table 43 also compares the chlorophyll/P ratios for 1971-1978 with various concentrations of *Daphnia* and other herbivorous zooplankters. Comparison of the chlorophyll/P ratio with all herbivores gives an r value of +.34, clearly inconsistent with the phenomenon of grazing. However, a comparison of the ratio with *Daphnia galeata* concentrations gives an r value of -.36, and comparison with all *Daphnia* together gives an r of -.58. Interestingly *Daphnia retrocurva* must be an important grazer along with *Daphnia galeata* as the addition of *Daphnia retrocurva*, the chief "other" *Daphnia*, raises the r value considerably.

One problem with this analysis is the high chlorophyll/P ratio in 1975 when, as the data in Table 41 show, the mean *Daphnia* population was large (150,000 m-2) and presumably, therefore, grazing rates were high. However, as the data in Table 41 show, the concentrations of *Daphnia* on July 1, 1975, were exceptionally high and if these are omitted the mean becomes much less

Table 42: Relationship between surface concentrations of chlorophyll and total phosphorus in L. Harriet, 1971-1978.

	July-Sept. Mean (mg . m^{-3})	
Year	Chl.	TP
1971	3.1	42
1972	3.5	38
1973	3.9	38
1974	29.0	50
1975	14.0	36
1976	3.6	27
1977	4.8	32
1978	7.2	37

All data, r = 0.685

Minus 1974 and 1975, r = -0.57

Table 43: Chlorophyll/phosphorus ratios in L. Harriet 1971-1978 compared with those in Wirth L. 1974-1978; relationship of chl/p ratios in L. Harriet to various groupings of grazers.

	mean chl/TP July-Sept.		mean concentrations of various grazing cladocerans, thousands m^{-2}, July-Sept.		
Year	Harriet	Wirth	Daphnia Ceriodaphnia & Diaphanosoma	All Daphnia	D. galeata
1971	.073		239	110	74
1972	.092		302	188	104
1973	.105		--	--	--
1974	.592	.930	291	32	28
1975	.413	.430	296	150(56)	50(2)
1976	.135	.670	265	154	22
1977	.156	.490	184	91	38
1978	.210	.170*	145	92	0

* = year following rotenone treatment of Wirth L.

( ) = value without data from July 1, 1975

r chl/TP vs. Daphnia + Ceriodaphnia + Diaphanosoma = +.34

r chl/TP vs. all Daphnia = -.58 (-.82)

r chl/TP vs. D. galeata = -.36 (-.51)

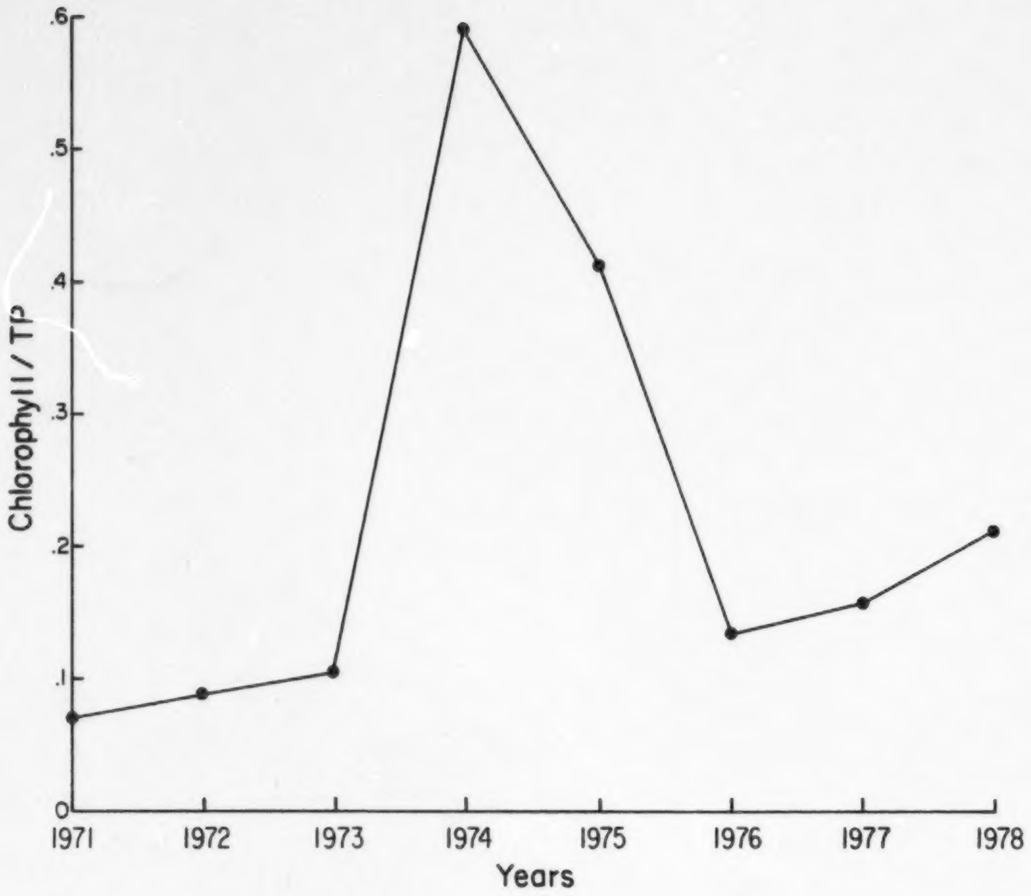

Fig. 90. Mean summer values for the ratio chlorophyll/TP in L. Harriet surface waters, 1971 - 1978.

(56,000 m-2) which is in much more agreement with the other results. Then, the r values for correlation of chlorophyll/P ratios with *Daphnia galeata* and with all *Daphnia* are -.51 and -.82 respectively. Again addition of *D. retrocurva* betters the correlation significantly.

It is interesting to note (Table 43) that the chlorophyll/P ratios in Lake Harriet for 1971-1973 were similar to those in Wirth Lake in 1978 i.e. when herbivores became abundant in Wirth Lake as a result of rotenone treatment the year before. Furthermore the 1973 and 1975 ratios in Lake Harriet were similar to those in Wirth Lake before its rotenone treatment.

Further evidence of the "grazing" hypothesis comes from the chlorophyll/phaeophytin ratios found in Lake Harriet in 1977 which suggests fairly convincingly (r2 = 0.63, r = 0.79) that the normally low concentrations of chlorophyll in the surface waters of Lake Harriet are accompanied by high percentages of phaeophytin -- a phenomenon consistent with the high grazing rate.

Thus it appears that the high concentrations of chlorophyll in Lake Harriet in 1974 and 1975 probably resulted from the unusually low concentrations of herbivorous zooplankters in those years.

The question then arises, why were the herbivores less abundant during those two summers? According to the Department of Natural Resources fish survey data, the perch population had been declining from 1964 to 1974 and probably, therefore, selective predation on the larger herbivores had been declining as well. Thus one would have expected that in 1974 herbivores would have been at a peak, or at least more abundant than in 1971 and 1972. However, this assumes that *abundance* of the fish is the sole indicator of their importance in predation and clearly this is not so. The degree of predation must depend on the spatial relationship between the fish and their prey -- the *Daphnia*. If the *Daphnia* had a refuge from the fish they would face less predation, but if the refuge were to vanish, their numbers could decline. This may be what happened in Lake Harriet. The dissolved oxygen data show, for the years when it is available, that in 1974 and 1972 and to some extent in 1978, when herbivores were relatively abundant, the upper part of the hypolimnion maintained higher dissolved oxygen concentrations than during 1974 and 1975 when the herbivores were least abundant (Fig. 89). That is, in 1974 and 1975 the refuge vanished when the *Daphnia*, unable to survive in the lower colder waters because of the low oxygen content, were forced to move upwards into the warmer epilimnion where predation by warm water reduced their numbers.

Once again, therefore, the question is, why did the oxygenated refuge vanish? Increased concentrations of total phosphorus in 1974 may provide the explanation. Primary production is directly related to total phosphorus (Smith 1979) and hypolimnetic oxygen depletion is directly related to primary production. Thus the decrease in dissolved oxygen very likely resulted from the increase in total phosphorus in 1974. Although the total phosphorus in 1975 was not high the oxygen deficit was still such that the refuge for the *Daphnia* did not exist.

The explanation detailed above, if proven true, would probably be the first demonstration of a *threshold* effect in eutrophication, or at least in the response of a lake to a nutrient. It would not have been expected from the empirical relationships available to us from the work of Dillon and Rigler (1974) or Jones and Bachman (1976) that such a small increase in phosphorus could cause such a large increase in algal biomass. However, considering the biological interaction makes it not only possible but likely that this did happen. It would be worth testing this idea in other lakes, even in Lake Harriet itself, by adding sufficient nutrients to increase depletion of dissolved oxygen in the upper part of the hypolimnion. As the amount of phosphorus reaching Lake Harriet from "normal" sources is in the range of 800 kg yr-1, the amount that would have to be added is within practical limits.

The reason for the high chlorophyll/P ratio in 1975 may have been the carryover of the 1974 oxygen deficit. However, another reason may lie in an increased number of perch in the lake, following the removal of their predators, and the inability of the introduced muskellunge to control the perch. These changes are reflected in the higher chlorophyll/P ratios from 1976-1978 than from 1971-1972. Because of the vulnerability of *Daphnia* to predation by perch, disappearance of their refuge under these conditions of high perch populations could result in a dramatic increase of chlorophyll in Lake Harriet. Already mean chlorophyll values in 1977 and 1978 are higher than for any years except for 1974 and 1975.

One difficulty with the scheme as outlined is that one would have expected the *Daphnia* to be smaller as well as rarer in 1974 and 1975. However, this does not seem to have happened. Perhaps under certain circumstances predation may be non size-selective.

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VI. CONCLUSIONS AND RECOMMENDATIONS

In previous publications (Shapiro, et al. 1975, Shapiro, 1980a, 1980b) we have attempted to convince those involved in lake restoration to think in ecological terms. That is, we have urged utilization of trophic relationships existing within lakes as adjuncts or alternatives to nutrient control. The work presented in this report reinforces our belief that such an approach -- biomanipulation -- could be feasible and effective. We believe that by limiting lake restoration to nutrient control, we are abrogating our role as ecologists and making it impossible to restore many lakes which might otherwise be benefited. Clearly, we do not have all the answers. We do not know whether such unexpected consequences as the appearance of Aphanizomenon following planktivore reduction will be common, and we do not know the duration of effectiveness of any of the measures proposed. However, such measures as rotenone treatment to eliminate planktivores and/or benthivores, stocking of piscivores, and formation of zooplankton refuges are generally much less expensive than the measures for nutrient reduction, and should be investigated before such expensive ventures are entered into. Similarly, changes that might dramatically reduce algal abundance, such as increases in nutrient loading sufficient to eliminate zooplankton refuges, and stocking with planktivores, should be avoided. Recent deliberate whole-lake manipulations such as those of Benndorf et al. (1981) and Shapiro and Wright (1982) are encouraging. We hope this report will stimulate others to attempt lake restoration by biomanipulation.

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